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(71) Applicant and
(72) Inventor: **GUPTA, Ajay** [US/US]; 39151 Horton Drive, Farmington Hills, MI 48331 (US).

(74) Agent: **MONACO, Daniel, A.**; Seidel, Gonda, Lavorgna & Monaco, P.C., Suite 1800, Two Penn Center Plaza, Philadelphia, PA 19102 (US).

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(54) Title: METHOD AND PHARMACEUTICAL COMPOSITION FOR PARENTERAL ADMINISTRATION OF IRON

(57) **Abstract:** Methods and compositions for the parenteral administration of iron are provided, inclusive of administration via hemodialysis or peritoneal dialysis solutions during the process of dialysis. The iron is in the form of a monomeric, noncolloidal iron compound suitable for parenteral administration.

METHOD AND PHARMACEUTICAL COMPOSITION FOR PARENTERAL ADMINISTRATION OF IRON

Field of the Invention

The invention relates to methods and compositions for the therapeutic delivery of iron to patients in need of iron supplementation.

Background of the Invention

Therapeutic Iron Administration

Iron deficiency is the most common nutritional problem worldwide, causing iron deficiency anemia in 500-600 million people (Cook, Skikne & Baynes, 1994; DeMaeyer & Adiels-Tegman, 1985). Iron deficiency is associated with prematurity and low birth weight during pregnancy, defects in cognitive and psychomotor development during childhood, and impaired work capacity in adulthood (Basta, Soekirman, Karyadi & Scrimshaw, 1979; Lieberman, Ryan, Monson & Schoenbaum, 1988; Lozoff, Jimenez & Wolf, 1991; Ohira et al., 1979; Oski & Honig, 1978; Oski, Honig, Helu & Howanitz, 1983). Oral iron supplementation programs have failed primarily due to patient noncompliance and gastrointestinal adverse effects (Schultink, van der Ree, Matulessi & Gross, 1993).

As an adjunct or alternative to the oral route, iron has been administered parenterally for more than one hundred years (Stockman,

1893). Patients that may benefit from parenteral iron may have iron deficiency from diverse causes including a) nutritional deficiency; or b) blood loss secondary to cancer, gastrointestinal ulceration, gynecological causes, extracorporeal blood loss in hemodialysis patients, or worm (e.g. hookworm) infestation.

Simple iron salts are considered too toxic for parenteral administration, since ionization of these compounds liberates free iron, and iron is a transition element capable of catalyzing free radical generation and lipid peroxidation (Brown, Moore, Reynafarje & Smith, 1950; Minotti & Aust, 1992). Of the two common valences, Fe(II) is the most reactive form leading to the production of highly reactive hydroxyl radicals by the Fenton reaction, or alkoxyl and peroxyl radicals from the breakdown of lipid peroxides (reviewed by Gutteridge and Halliwell, 1990).

Therefore only colloidal iron compounds, that are polynuclear ferric hydroxide carbohydrate complexes, are currently in use for parenteral administration of iron. These compounds are characterized by a complex structure in which a core of ferric iron lies surrounded by a complex carbohydrate structure, so that iron core is shielded from coming in direct contact with plasma or cells. Examples of these compounds include iron dextran, polymaltose, gluconate, saccharate and chondroitin sulfate. These polynuclear iron complexes have very low intravenous acute toxicity (LD_{50} > 200 - 2,500 mg Fe/kg), compared to soluble iron salts (10 - 20 mg Fe/kg), attributed to their low ionic iron content, since most of the iron is present as Fe(III) that is bound strongly to the carbohydrate moiety (Geisser, Baer & Schaub, 1992). However colloidal iron compounds are also associated with serious side effects including hypotension and anaphylactoid reactions (Goldberg, 1958). The toxicity of these compounds may be secondary to liberation of free iron due to chemical interactions in plasma (Goldberg, 1958), or due to the presence of free iron in the pharmaceutical preparation.

For instance, the ionic iron content of an iron dextran preparation (Imferon®) was approximated to be 1/300th of the total iron present (Fielding & Smith, 1963). Furthermore, Cox and coworkers have found that 1-2% of iron present in fresh ampules of Imferon® is ferrous iron, present as an extremely weak ferrous-dextran complex (Cox, King & Reynolds, 1965). Presumably, depolymerization of iron-dextran complex releases free dextran molecules (Mol. Wt. ~ 6000 Dalton, ~ 33 glucose units) and ionic iron. Furthermore, change in pH when the polynuclear iron complexes come in contact with plasma may further induce depolymerization and formation of ferric hydroxide (Goldberg, 1958). Consistent with these in vitro results, a recent clinical study found that 8 of the 10 hemodialysis patients given 100 mg Fe(III) hydroxide sucrose complex intravenously had bleomycin detectable free iron in the circulation (Banyai et al., 1998). Acute adverse reactions to intravenous iron dextran occur at a frequency of six to seven episodes per 1,000 hemodialysis patients treated (Fishbane et al., 1996). In extreme cases, refractory hypotension, respiratory failure and death may ensue. These reactions have been attributed to release of free iron in the circulation. In the anesthetized cat, infusion of iron dextran complex (Imferon®) produces a hypotensive response that exhibits a rough correlation with its ferrous iron content and can be abolished by reducing the speed of infusion, while ferric iron produces a smaller transient depressor response followed by a more sustained presser response (Cox et al., 1965; Goldberg, 1958). That redox active iron is released by colloidal iron compounds in the circulation is further evidenced by the rise in plasma total peroxide and malondialdehyde concentrations within ten minutes following infusion of 100 mg iron sucrose complex (Roob et al., 1998). The free radical generation and lipid peroxidation catalyzed by free iron may also play a role in the pathogenesis of a variety of chronic diseases such as inflammation, ischemia, atherosclerosis, cancer, heart disease, and stroke. Recent evidence suggests an increase in cardiovascular and infectious mortality

in the US maintenance dialysis patients receiving higher doses of iron dextran intravenously (Collins, Ebben, Ma & Xia, 1998).

The carbohydrate complex, such as the dextran moiety, released by depolymerization of colloidal iron compounds may also be immunogenic, thereby stimulating formation of circulating antibodies. Circulating anti-dextran antibodies in patients administered repeated doses of iron dextran have also been implicated in anaphylactoid reactions (Cox et al., 1965).

Colloidal iron complexes, when introduced directly into the circulation, are unable to efficiently donate their iron to apotransferrin, to form transferrin. Hereinafter, the term "transferrin" shall be used to refer to both the precursor form of the protein which lacks iron, and the iron bound form. These colloidal iron complexes are ideally deposited in the reticuloendothelial system (RES) after parenteral administration, processed in the RES, and then iron is released into the circulation where it binds to apotransferrin and is transported to the target tissues. Iron-dextran complex (INFeD) is an example of such a colloidal iron complex. Only about 50% of iron delivered as these colloidal complexes is available and utilized for hemoglobin generation. The fate of the rest is not known. It is possible that the rest of the iron administered parenterally lies entrapped in cells, and is not bioavailable. It is conceivable that this entrapped iron leads to oxidant stress. Complexes that also go to the parenchymal cells such as hepatocytes and myocardial cells, in addition to the RES, cause cellular damage and should not be administered parenterally.

For parenteral administration it would be desirable to use iron compounds that are not processed in the reticuloendothelial system and instead can donate their iron directly to transferrin. Parenteral administration of monomeric iron salts or chelates makes iron available in the circulation without the need for processing by reticulo-endothelial system. However these compounds have hitherto been considered too toxic for parenteral use for the following reasons.

First, monomeric iron compounds such as sulfate, gluconate, and ascorbate readily dissociate in body fluids, releasing free iron. The reaction of Fe (III) aquo ion with plasma proteins due to its high positive charge, results in denaturation of proteins and partial precipitation. The Fe(II) species is the more reactive form. As indicated above, Fe(II) leads to the production of highly reactive hydroxyl radicals, or alkoxyl and peroxyl radicals.

Second, the rate at which iron is donated to transferrin by an iron compound is highly dependent on the nature of the iron compound. If the compound is not able to efficiently donate iron to transferrin, the complex or the iron present in it may bind nonspecifically to a variety of plasma proteins, including albumin. It is this fraction that is rapidly taken up by tissues nonspecifically and causes toxic reactions.

For the above reasons, the LD_{50} for monomeric iron compounds such as ferrous gluconate and sulfate is only about 11 mg Fe/kg body weight in white mice, compared with more than 2500 mg Fe/kg body weight for polynuclear iron-carbohydrate complexes, after intravenous administration (Geisser et al., 1992).

Therefore, only colloidal polynuclear/polymeric iron complexes are currently available for parenteral use. However as reviewed above, use of these compounds is associated with significant toxicity, morbidity and mortality. Furthermore, the chemical process required to synthesize polynuclear iron complexes is complex and cumbersome thereby making these drugs expensive. The side effects and expense associated with polymeric iron complexes have limited their use in clinical practice. Iron deficiency anemia is the most common nutritional deficiency worldwide, and oral iron compounds have had a very limited impact on the problem because of their gastro-intestinal side effects. Therefore there is a great need to make available monomeric iron salts, complexes and chelates (hereafter referred to as monomeric iron compounds) that may be suitable for parenteral administration to mammals by virtue of being devoid

of the problems associated with polymeric iron complexes (such as iron dextran or iron gluconate) or the known monomeric iron compounds (such as ferrous sulfate, iron ascorbate, ferrous gluconate, and ferric citrate).

Iron Deficiency in Hemodialysis and Peritoneal Dialysis Patients

Patients with chronic renal failure are treated by dialysis. Dialysis is required to maintain homeostasis in patients with end stage kidney failure. Dialysis is defined as the movement of solute and water through a semipermeable membrane which separates the patient's blood from the dialysate solution. The semipermeable membrane can either be the peritoneal membrane in peritoneal dialysis patients or an artificial dialyzer membrane in hemodialysis patients.

Patients with chronic renal failure suffer from anemia due to impaired production of erythropoietin (Erslev, 1991). Clinical manifestations of chronic renal failure improve as uremia and volume overload are corrected by dialysis. However, anemia due to lack of erythropoietin becomes a major limiting factor in the functional well being of end stage renal disease patients.

Molecular cloning of the erythropoietin gene (Jacobs, et al., 1985) led to commercial production of recombinant erythropoietin, which was a major advance in the treatment of renal anemia (Erslev, 1991; Levin, 1992). Erythropoietin therapy functions by stimulating red cell production and thereby iron utilization. With the use of erythropoietin therapy, transfusions are avoided in most chronic dialysis patients. Blood tests and gastrointestinal bleeding further contribute to loss of iron. Therefore, accelerated iron utilization coupled with small but unavoidable loss of extra corporeal blood with hemodialysis and increased gastrointestinal losses of iron lead to iron deficiency in almost all patients on long term maintenance dialysis.

Other factors that may contribute to an iron deficient state are the restricted renal diet which may be deficient in iron, and iron absorption

may be impaired by uremia per se. Co-administration of additional medications, such as phosphate binders with food, may also impair iron absorption. Therefore, iron deficiency has become a major problem in the dialysis patients treated with erythropoietin.

In clinical practice, transferrin saturation (ratio of serum iron to total iron binding capacity) and serum ferritin are used to assess the iron status. The majority of maintenance dialysis patients receiving erythropoietin therapy can be arbitrarily classified into six groups depending on their iron status (Table 1, below).

In states of iron deficiency, iron supply to bone marrow is not maintained and the response to erythropoietin is impaired. Indeed, iron deficiency is the most common cause of erythropoietin resistance (Kleiner, et al., 1995). Uremic patients suffering from absolute or functional iron deficiency require lower doses of erythropoietin if they receive effective iron supplementation. Based on these considerations, Van Wyck, et al., (1989) have suggested that all renal patients with low to normal iron stores should prophylactically receive iron. Iron supplementation is accomplished most conveniently by the oral administration of iron one to three times a day.

Table 1. Iron Status in End Stage Renal Disease

Iron status	Serum Fe/TIBC (TSAT)	Serum Ferritin (µg/L)
Severe iron deficiency	< 15%	< 50
Moderate iron deficiency	15-17%	50-100
Mild iron deficiency	18-25%	100-200
Optimal iron status	25-50%	200-800
Iron overload	> 50 %	> 800
Reticuloendothelial block	< 20%	> 500

A problem exists because oral iron is often not tolerated due to gastrointestinal side effects. Practical problems such as noncompliance, impaired absorption when taken with meals, and other factors are further combined with the problem of tolerating oral iron. Oral iron is also ineffective due to impaired iron absorption. Macdougall, et al. (1989) also found a retarded response to recombinant human erythropoietin in hemodialysis patients on oral iron, which was corrected once iron was given intravenously. Schaeffer and Schaeffer (1995), have recently demonstrated that only intravenous but not oral iron, guarantees adequate marrow iron supply during the correction phase of recombinant erythropoietin therapy.

In Europe, iron is available for intravenous administration as iron dextran, iron saccharate and iron gluconate. In the United States, iron dextran is approved for intravenous use and is widely used for this purpose in dialysis patients. However, there are controversies with regard to the dosage and frequency of injection.

On the one hand, intravenous iron therapy has several advantages over oral administration. Intravenous therapy overcomes both compliance problems and the low gastrointestinal tolerance often observed in patients on oral therapy. Schaefer and Schaefer (1992) reported a 47% reduction in erythropoietin dose when intravenous iron was given to iron deficient hemodialysis patients previously treated with oral iron. On the other hand, intravenous iron therapy with compounds presently in use does have risks and disadvantages. Anaphylactoid reactions have been reported in patients (Hamstra et al., 1980; Kumpf et al., 1990). Therefore, a test dose must be administered when parenteral iron therapy is first prescribed. Intravenous iron therapy can also cause hypotension, and loin and epigastric pain during dialysis which may be severe enough to stop the treatment. Further, the intravenous drug is expensive and requires pharmacy and nursing time for administration. With intravenous iron therapy, serum iron, transferrin and ferritin levels must be regularly

monitored to estimate the need for iron and to measure a response to the therapy. Finally, there is also a concern about potential iron overload with intravenous therapy, since the risk of infection and possibly cancer are increased in patients with iron overload (Weinberg, 1984). Recent evidence further suggests a 35% higher risk for cause-specific infectious deaths in US Medicare ESRD patients given intravenous iron frequently (Collins et at., 1997).

In view of the above, neither the oral nor intravenous iron therapy route with the presently utilized compounds is ideal. Alternative compounds and routes of iron administration are desirable, particularly for dialysis patients. The hypotensive effects of intravenous iron dextran are completely abolished, irrespective of the total dose administered, by reducing the rate of infusion or by preliminary dilution of the iron dextran with isotonic saline (Cox et al., 1965). Addition of an iron compound to the hemodialysis or peritoneal dialysis solutions could lead to a slow transfer of iron into the blood compartment if the dialysis membrane is permeable to the iron salt. However, colloidal iron compounds or iron in its mineral form are not soluble in aqueous solutions and therefore not suitable for addition to the dialysate. Furthermore, iron is known to be toxic when administered parenterally in its mineral form. The toxic effects may arise from precipitation of iron in the blood, producing multiple pulmonary and sometimes systemic emboli. Symptoms resembling that of fat embolism occur. Irritation of the gastrointestinal tract gives rise to diarrhea and vomiting. Also, depression of the central nervous system can lead to coma and death (Heath et al., 1982).

There are currently no soluble, non-colloidal, monomeric compounds suitable for parenteral administration. Because of the numerous problems associated with polymeric, colloidal complexes, it would be highly advantageous to identify, test and make available monomeric iron compounds that are safe and effective when given parenterally.

Recent studies have shown that polyphosphate compounds are possible candidates for intracellular iron transport (Konopka et al., 1981; Pollack et al., 1985). Among these polyphosphate compounds, pyrophosphate has been shown to be the most effective agent in triggering iron removal from transferrin (Pollack et al., 1977; Morgan, 1979; Carver et al., 1978). Pyrophosphate has also been shown to enhance iron transfer from transferrin to ferritin (Konopka et al., 1980). It also promotes iron exchange between transferrin molecules (Morgan, 1977). It further facilitates delivery of iron to isolated rat liver mitochondria (Nilson et al., 1984). Ferric pyrophosphate has been used for iron fortification of food and for oral treatment of iron deficiency anemia (Javaid et al., 1991).

Ferric pyrophosphate has also been used for supplying iron to eukaryotic and bacterial cells, grown in culture (Byrd et al., 1991). Toxic effects of ferric pyrophosphate have been studied by Maurer and coworkers in an animal model (1990). This study showed an LD_{50} slightly higher than 320 mg of ferric pyrophosphate per kilogram or approximately 30 milligrams of iron per kilogram body weight. The effective dose for replacing iron losses in hemodialysis patients is estimated to be 0.2 to 0.3 milligrams iron per kilogram body weight per dialysis session. Therefore, the safety factor (ratio of LD_{50} to effective dose) is over 100.

Another metal pyrophosphate complex, stannous pyrophosphate has been reported to cause hypocalcemia and immediate toxic effects. Since ferric ion forms a stronger complex to pyrophosphate than do stannous ion, or calcium ion (Harken et al., 1981; Sillen et al., 1964), hypocalcemia is not a known side affect of ferric pyrophosphate administration.

The U.S. Patent 4,76,838 to Veltman, issued July 12, 1988, discloses a dry, free flowing, stable, readily soluble, noncaking, particulate soluble products which are readily soluble in water and are useful for preparing solutions for use in hemodialysis. The patent discloses the fact that currently used dialysis procedures do not ordinarily take into account

those materials in blood that are protein bound. Examples are iron, zinc, copper, and cobalt. The patent states that it is an object of the invention to make such materials as an integral part of dry dialysate products. However, no specific disclosure is made on how to make the iron available through the hemodialysis. Furthermore, no direction is given towards a soluble versus colloidal, monomeric versus polymeric, ferrous versus ferric, iron compound that may be suitable for administration via the dialysate. Also, no discussion is provided concerning the nature of specific ligands to which the iron is complexed in the iron compound.

What are needed are methods and physico-chemical criteria which permit the successful selection of iron compounds, particularly monomeric Fe (III) compounds, that are suitable for parenteral administration, including administration via dialysate. In particular, methods and criteria are needed for the selection of iron compounds that are free of the problems associated with the use of polymeric colloidal iron complexes.

Summary of the Invention

According to the present invention, physico-chemical criteria and methods are described for the successful selection of monomeric Fe (III) compounds that are suitable for parenteral administration. Clinical trial of one such compound (soluble ferric pyrophosphate) in dialysis patients is described. Clinical scenarios and methods of administration of these compounds to patients by the intravenous, intramuscular, subcutaneous and transdermal routes or by addition to dialysate in dialysis patients (collectively referred to as "parenteral") are described. Lastly, other novel approaches that may be used to mitigate the iron toxicity are described.

In one aspect, the invention is a pharmaceutical composition comprising a pharmaceutically acceptable carrier suitable for parenteral administration and a non-toxic, monomeric iron compound. The iron compound comprises one or more iron atoms bound to one or more ligands, which iron compound:

(a) is suitable for parenteral administration to mammals, for the treatment of iron deficiency;

(b) is able to donate a substantial portion of its iron content directly to the protein transferrin under physiological conditions;

(c) does not induce significant binding of the contained iron to proteins other than transferrin, or to other ligands found in body fluids, under physiological conditions;

(d) does not contain a ligand which significantly complexes with metal ions normally present in body fluids, under physiological conditions;

(e) does not release a clinically significant amount of free iron to body fluids, under physiological conditions; and

(f) has a molecular weight of less than about 30,000 daltons.

In another embodiment, a method of treating iron deficiency in a mammal comprises parenterally administering to the mammal an effective amount of the aforesaid non-toxic, monomeric iron compound.

In another embodiment, a method of iron administration to a dialysis patient is provided comprising administering to the patient through a hemodialysis solution or peritoneal dialysis solution an effective amount of a non-toxic, monomeric iron compound comprising one or more iron (III) atoms bound to one or more ligands, which iron compound:

(a) is able to donate a substantial portion of its iron content directly to the protein transferrin under physiological conditions;

(b) does not induce significant binding of the contained iron to proteins other than transferrin, or to other ligands found in body fluids, under physiological conditions;

(c) does not contain a ligand which significantly complexes with metal ions normally present in body fluids, under physiological conditions;

(d) does not release a clinically significant amount of free iron to body fluids, under physiological conditions;

(f) has a molecular weight of less than about 12,000 daltons; and

(h) is water-soluble;

wherein the iron compound is infused into the circulation of the patient during dialysis by diffusion across a semipermeable membrane.

Where the dialysate is a hemodialysate, i.e., it is intended for hemodialysis, the iron concentration in the dialysate may range from about 1 to about 70 µg per deciliter. Where the dialysate is to be used for peritoneal dialysis, the iron concentration in the dialysate may range from about 1 to about 500 µg per deciliter.

Also contemplated is a dialysate concentrate containing the iron compound. Thus, for example, the iron concentration in a hemodialysate concentrate will range from about 0.3 to about 35 mg/L.

According to one embodiment, the iron compound is ferric pyrophosphate. In other embodiments, the compound is other than ferric pyrophosphate, such as hydroxamate or a hydroxypyridinone. Any of the compositions of the invention may optionally comprise one or more antioxidants.

In the therapeutic administration of iron compound according to the present invention, iron is preferably delivered to the circulation of the mammal at a rate such that the iron binding capacity of transferrin in the body of the mammal is not exceeded by the delivered iron. Most preferably, iron is delivered to the circulation of the mammal at a rate such that 80% of the iron binding capacity of transferrin in the body of the mammal is not exceeded by the delivered iron.

The optional antioxidant is administered proximal in time to the administration of the iron compound. Hence, the antioxidant may be administered simultaneously with the iron compound, or shortly before or after the iron compound. Preferably, the antioxidant is combined with the iron compound in the same formulation.

Description of the Figures

FIGURE 1 is a pair of graphs showing serum iron versus time and iron per TIBC (percent) versus time.

FIGURE 2 is a graph showing serum iron per total iron binding capacity (TIBC) (percent).

FIGURE 3 is a graph showing the study design and concentration of iron in the dialysate over the study period.

FIGURE 4 is a graph of group whole blood hemoglobin average over the study period.

FIGURE 5 is a graph of the group reticulocyte hemoglobin average amount over the study period.

FIGURE 6 is a graph of the group predialysis serum iron level average over the study period.

FIGURE 7 is a graph of the group increment in average serum iron with dialysis over the study period.

FIGURE 8 is a graph of the group predialysis total iron binding capacity average over the study period.

FIGURE 9 is a graph of the group predialysis transferrin saturation (TSAT) average over the study period.

FIGURE 10 is a graph of the group postdialysis transferrin saturation (TSAT) average over the study period.

FIGURE 11 is a graph of the group average change in transferrin saturation (TSAT) during dialysis over the study period.

FIGURE 12 is a graph of the group average percentage change in mean transferrin saturation (TSAT) with dialysis over the study period.

FIGURE 13 is a graph of the group predialysis ferritin average over the study period.

FIGURE 14 is a graph of the group erythropoietin dose per treatment average over the study period.

FIGURE 15 is a graph of the group weekly dose of intravenous iron (Infed®) average over the study period.

FIGURE 16 is a graph showing the serum iron in rabbits undergoing acute peritoneal dialysis with a dialysis solution that contains ferric pyrophosphate.

FIGURE 17 is a graph showing total iron binding capacity (TIBC) in rabbits during peritoneal dialysis.

FIGURE 18 is a graph showing the transferrin saturation (serum Fe/TIBC, %) in rabbits undergoing acute peritoneal dialysis with a dialysis solution that contains ferric pyrophosphate.

FIGURE 19 is a graph of serum iron (μg/dL) and transferrin saturation (TSAT%) as a function of time in a human hemodialysis patient receiving ferric pyrophosphate via the dialysate.

Detailed Description of the Invention

According to the present invention, monomeric iron compounds are administered safely and effectively by parenteral administration for the prevention or treatment of iron deficiency in a mammal. By "monomeric" is meant a compound which is not an oligomer or polymer. Thus, the iron compound is mononuclear, as opposed to polynuclear. By "iron compound" is meant not only simple salts of iron, but also all other associations of iron atoms with other atoms or molecules, e.g., complexes or chelates of iron. The compound may be organic or inorganic in nature. The compound is composed of iron atoms and another atom or chemical group which may be an anion (as in the case of an iron salt) or a chelate or other ligand, which is typically organic in nature. The term "ligand" shall be used herein to mean all such atoms and chemical groups which may form an iron compound as defined above.

The successful prediction of biological activity, safety and efficacy for a given iron compound depends on an understanding of its function in biological systems and the ability to relate these functions to

simple physicochemical properties of the iron compound measured in vitro. The iron compounds utilized in the practice of the invention are characterized by the hereinafter-described properties.

The iron compound utilized in the practice of the present invention may reside in the iron (II) (ferrous) or iron (III) oxidation state, but are preferably in the iron (III) state at the time of administration into the mammal.

The iron compound must be able to donate its iron rapidly to transferrin. Transferrin is the major carrier of iron in mammalian systems and is responsible for iron transport to the target tissue. Fe(III) acetohydroxamate is an example of an iron compound which is able to rapidly donate its iron to transferrin. The iron compound should thus be able to donate a substantial portion of its iron content directly to transferrin under physiological conditions. By "substantial portion" is meant at least about 50% of the iron content of the iron compound.

The iron compound should not induce significant binding of the contained iron to proteins other than transferrin, or to other ligands found in body fluids, under physiological conditions. By "significant binding" with respect to the amount of iron binding to ligands other than transferrin is meant the proportion of a therapeutic iron dose that when it complexes to other ligands or proteins produces clinically significant side effects.

The ligand should not significantly complex with other metal ions present in body fluids such as calcium, magnesium, zinc and copper. By "significantly complex" is meant that the ligand induces a more than about 10% reduction in the serum concentration of the aforesaid other metal ions, under physiological conditions.

As an arbitrary generalization, a 10% change in the value of a clinical parameter is considered clinically significant, since the impreciseness of a laboratory test used to measure a parameter is usually less then 10% of the actual value.

The ligand comprising the iron compound should be strongly complexed to the iron atom, preferably Fe(III), such that when the iron compound is administered in therapeutic doses it does not release a clinically significant amount of free iron to the patient's body fluids, under physiological conditions. By "clinically significant amount" is meant an amount of free iron which would result in significant oxidant stress to the patient, or any other side-effects as hereinafter discussed. Methods for determining free iron concentrations which are responsible for oxidant stress are known to those skilled in the art. Oxidant stress induced by iron administration according to the present invention is minimal.

Preferably, the iron compound is characterized by a log of conditional stability constant (as hereinafter described) of at least 6 in a physiological electrolyte solution.

The iron compound and the ligand should be biocompatible and safe for administration to humans or animals, particularly administration by the parenteral route. Ferric pyrophosphate is an example of such a compound.

The iron compound has a molecular weight of less than about 30,000 daltons for intravenous administration. For dialysis administration, the molecular weight should be preferably less than about 12,000 daltons, more preferably less than about 10,000 daltons.

An additional property that may be advantageous but not absolutely necessary is that the ligand should be able to extract iron from ferritin, the iron storage protein. Acetohydroxamate is an example of such a ligand.

The monomeric iron compounds that are highly stable, possess iron which is not available for transfer to transferrin, can be considered similar to the polynuclear iron-carbohydrate complexes. Such compounds are not believed suitable for parenteral administration, and it is not the purpose of this invention to make these compounds available for parenteral use. However, it should be noted that such highly stable

compounds may be very desirable for oral administration because local release of free ionic iron in the gastrointestinal tract by iron salts is primarily responsible for the oxidant damage, inflammation, ulceration and clinical gastrointestinal toxicity. Stability of these compounds will prevent dissociation and release of free iron. Furthermore, even though iron in these compounds may not be available for transfer to transferrin directly, the processing of these compounds in the intestinal mucosal cells may make this iron bioavailable.

According to one embodiment of the invention, the monomeric iron compound is ferric pyrophosphate. According to another embodiment, the compound is other than ferric pyrophosphate.

Methods to predict and identify mononuclear iron compounds that are potentially suitable for parenteral administration

Parenteral administration of iron compounds according to the present invention delivers the compounds directly into the circulation when administered intravenously or via the dialysate, and into the interstitial fluid when transdermal, subcutaneous or intramuscular routes are used. To test the suitability of candidate iron compounds, laboratory experiments done in vitro are conducted under conditions that mimic electrolyte composition of human body fluids such as plasma and interstitial fluid. Therefore, a suitable solution to test the stability of candidate iron compounds consists of about 140 mM sodium, 4 mM potassium, 1.2 mM calcium, 0.9 mM magnesium, 1.5 micromolar zinc, 1.6 micromolar copper, 100 mM chloride, 1 mM lactate and 1 mM inorganic phosphorus, and has a pH of about 7.4.

The following tests are performed to characterize the properties that identify candidate iron compounds useful in the practice of the present invention.

State of aggregation

The state of aggregation is determined to ensure that the compound is monomeric. This can be determined by testing the magnetic moments of a solution of the compound according to known procedures (Brown et al., 1978).

Kinetics of iron transfer

The kinetics of iron transfer from the monomeric iron compound to transferrin can be tested by mixing the iron compound with transferrin in vitro, and measuring the change in the transferrin absorption spectra using spectrophotometric techniques or nuclear magnetic resonance techniques as described by Brown and coworkers (Brown et al., 1978).

Ligand induced extraction of iron from ferritin

To test the ability of the ligand of the monomeric iron compound to extract iron from ferritin, a mixture of ferric citrate polymer and the ligand is incubated under physiological conditions. The rate of appearance of the Fe(III)-ligand complex is monitored using spectrophotometric techniques (Brown et al., 1978).

Stability

Determination of the stability of the iron compound in solution is achieved by characterizing the precise species distribution by means of analytical potentiometry and subsequent mathematical analysis of the potentiometric data by the method of Sarkar and Kruck (Sarkar & Kruck, 1973). According to this method, it may be shown that Fe(III)-acetohydroxamic acid is highly stable over the pH range 5.5-9, which includes the normal physiological pH range. Moreover, at pH=7, the species Fe(acetohydroxamic acid)$_3$ represents over 95% of the total species distribution (Brown, Chidambaran, Clarke & McAleese, 1978).

Conditional stability

Stability may be further determined with resort to the calculation of the conditional stability constant (β′) of the candidate compounds under physiological conditions.

Inverse values of dissociation (instability) constants are stability constants (β or K_{stab}). These constants are available from the literature for a large number of iron compounds, having been determined under non-physiologic conditions, often at extremes of pH and in the absence of any other competing ligands. However, when other competing ions are present, common side reactions as those caused by hydrogen ions, hydroxide ions, buffering substances, masking agents and disturbing metal ions occur. Therefore, the term 'conditional stability constant' was coined to stress that 'constant' is not constant but depends on experimental conditions.

Table 2 contains the conditional stability data for representative iron compounds containing the indicated ligands (AHA=acetohydroxamic acid; Des B= desferroxamine B; EDTA = ethylene diamine tetraacetic acid; DMPH=dimethyl hydroxypyridinone). Conditions "C" in Table 2 represent physiological conditions. The value of β′ under conditions "C" is herein defined as the "conditional stability constant" for a compound.

TABLE 2: Conditional Stability Constants (β') for the Equilibria $Fe' + xL' \rightleftharpoons FeL_x$ where $Fe' = Fe_{aq} + Fe(OH)_x + FeY$ (Y = phosphate, lactate, and Cl^-) and $L' = L + LH_x + ML_z$ ($M = K^+, Na^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}$)

Ligand (L)	Fe oxidation state	Species #	Log β' @ conditions A ‡	Log β' @ conditions B ‡	Log β' @ conditions C ‡
EDTA	+3	FeL ***	6.65	-3.45	-3.45
EDTA	+2	FeL	11.46	1.36	0.66
Sulfate	+3	FeL	-6.26	-6.47	-6.47
Sulfate	+2	FeL	2.20	1.99	1.29
Gluconate	+3	$Fe(OH)_3L$	17.03 ‡‡‡	17.02 ‡‡‡	17.02 ‡‡‡
Gluconate	+2	FeL	1.00	0.99	0.29
Succinate	+3	FeL_2 **	4.82	4.59	4.59
Succinate	+2	FeL ##	1.42	1.30	0.60
Fumarate	+2	FeL	2.78	2.78	2.08
Citrate	+3	FeL	2.27	-1.28	-1.28
Citrate	+2	FeL	4.79	1.24	0.54
Des B	+3	FeLH	17.42	12.09	12.09
Pyrophosphate	+3	$Fe(LH)_2$	13.64	9.30	9.30
AHA	+3	FeL_3 ###	13.94	12.81	12.81
AHA	+2	FeL	2.84	2.46	1.76
Catechol	+3	FeL_2	12.08	-1.17	-1.17
Catechol	+2	FeL	0.52	-6.11	-6.81
DMPH	+3	FeL_3	20.33	na ‡‡	na ‡‡
Glycine	+3	FeL	-0.67	-2.65	-2.65
Glycine	+2	FeL	1.96	-0.019	-0.72

Major species determined from distribution plot at the following conditions: pH 7.4; [Fe] = 1 μM, [L] = 10 μM.

‡ Conditional stability constant (β') for the equilibria $Fe' + xL' \rightleftharpoons FeL_x$ calculated at the conditions:

Conditions A (competing equilibria only involve H^+ and OH^-):

Fe not complexed to L present as $\{Fe^{n+} + Fe(OH)_x^{m+}\}$;

L not complexed to Fe present as $\{ L^{o-} + LH^{p-} + LH_2^{q-} + \text{etc}\}$

Conditions B (competing equilibria involve H^+, OH^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, and Zn^{2+}):

Fe not complexed to L present as $\{Fe^{n+} + Fe(OH)_x^{m+}\}$;

L not complexed to Fe present as $\{ L^{o-} + LH^{p-} + LH_2^{q-} + \text{etc} + NaL + KL + MgL + CaL + CuL + ZnL\}$ where $[Na^+] = 140$ mM, $[K^+] = 4$ mM, $[Mg^{2+}] = 0.9$ mM, $[Ca^{2+}] = 1.2$ mM, $[Cu^{2+}] = 1.6$ μM and $[Zn^{2+}] = 1.5$ μM.

Conditions C (competing equilibria involve H^+, OH^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, lactate, phosphate and Cl^-):

Fe not complexed to L present as $\{Fe^{n+} + Fe(OH)_x^{m+} + FeY\}$ where Y = lactate (1 mM), phosphate (1 mM) and Cl^- (100 mM);

L not complexed to Fe present as $\{ L^{o-} + LH^{p-} + LH_2^{q-} + \text{etc} + NaL + KL + MgL + CaL + CuL + ZnL\}$ where $[Na^+] = 140$ mM, $[K^+] = 4$ mM, $[Mg^{2+}] = 0.9$ mM, $[Ca^{2+}] = 1.2$ mM, $[Cu^{2+}] = 1.6$ μM and $[Zn^{2+}] = 1.5$ μM.

** 98% FeL_2 and 2 % FeL

98% FeL and 2% Fe_2L

*** 49% FeL, 51% FeL(OH)

60% FeL_3, 40% FeL_2

‡‡ Data for DMPH binding to Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+} not available.

‡‡‡ log ($\beta'/[H^+]^3$) at pH = 7.4 where $\beta' = [Fe(OH)_3L][H^+]^3 / \{ [Fe'][L'] \}$

The iron compounds of this invention are highly stable and largely undissociated under physiologic conditions, so that when introduced directly into the body fluids there is minimal dissociation of the compound before iron can be donated to transferrin. Consequently, there is minimal and clinically insignificant liberation of free iron. By definition, these compounds have high conditional stability constants, where the log of the constant is greater than at least 6.0 or 7.0. Fe(II) compounds are unlikely to meet this criteria. In fact only a few Fe(III) compounds meet this criteria as shown in Table 2. Furthermore, the conditional stability constant of iron for the ligand in the preferred monomeric iron compounds is high, and the iron (preferably ferric) is complexed to the ligand tightly such that there is no significant complexation of the ligand by the other metal cations present in body fluids.

The monomeric compounds used in the practice of the invention are characterized by a log of conditional stability constant greater than 6.0. Therefore, the compounds of this invention include, for example, Fe(III) complexed to acetohydroxamate and other hydroxamates; pyrophosphate; hydroxypyridinones; and derivatives of these compounds. By a "hydroxamate" is meant any compound which contains the hydroxamate group. By a "hydroxypyridinone" is meant any compound containing the hydroxypyridinone group. The conditional stability constant of dimethyl hydroxypyridinone (DMPH) could not be determined since the data for DMPH binding to sodium, potassium, magnesium, calcium, copper and zinc is not available. However the chemical structure and the high conditional stability constant when competing equilibria only involve hydrogen and hydroxyl ions (condition 'A', Table 2) suggests the suitability of this compound for the purposes of the invention. Compounds of this invention also include other known or yet to be discovered compounds with log β' values greater than 6.0. Furthermore, chelators of iron that function as natural siderophores or are their synthetic derivatives, may be suitable for

parenteral administration, since the siderophores have a high and specific affinity for Fe(III). Siderophore-Fe(III) complexes are involved in microbial iron transport. For a discussion of siderophores see P. Singleton and D. Sainsbury, *Dictionary of Microbiology and Molecular Biology*, 2d ed., John Wiley & Sons, New York, NY, 1987, pp 806-807, incorporated herein by reference. Generally, siderophores comprise low-molecular weight ferric iron-chelating compounds which are synthesized and exported by most organisms for the sequestration and uptake of iron. The two main structural classes of siderophores are the catecholamides and the hydroxamates.

Ferric pyrophosphate (log β' under physiologic conditions = 9.30) serves as a paradigm iron compound for the practice of the present invention. As hereinafter described, ferric pyrophosphate has been safely and effectively used by us for administration to dialysis patients, by its addition to the dialysate, and is therefore suitable for administration by other parenteral routes such as intravenous, intramuscular, subcutaneous, and transdermal.

Generation of free iron

The following is a practical method to determine the generation of free iron and complexation of other metal cations when an iron compound is administered parenterally. A physiologic amount of iron(III) compound is added to a solution containing transferrin (such as plasma). The amount of free iron generated is determined. The decrease in the serum concentration of uncomplexed or free metal ions of calcium, magnesium, zinc, etc. is also determined.

Accordingly, a plasma solution containing transferrin and an iron compound of interest, ferric pyrophosphate, was subjected to ultra-filtration to separate iron that is free in the plasma from the iron bound to transferrin. In this experiment, 3.5 liters of human plasma was subjected to hemodialysis in vitro using a F-80 polysulfone, high flux dialyzer and a

Fresenius-H hemodialysis machine. The plasma was dialyzed against bicarbonate dialysate containing 12 mcg/dl of iron, as ferric pyrophosphate. The dialysate flow rate was 800 ml/min, while the plasma flow rate was maintained at 300 ml/min. Plasma samples were taken prior to starting dialysis (pre-dialysis) and after 4 hours of hemodialysis (post-dialysis). These samples were subjected to ultrafiltration using an Amicon ultrafiltration membrane with a 10,000 molecular weight cut-off, thereby allowing free iron and its low molecular weight chelates, but not transferrin to go through the pores. Iron concentration was determined in the plasma samples and the samples of the ultrafiltrate using an atomic absorption assay. The pre-dialysis iron concentrations in the plasma and ultrafiltrate were 0.400 and 0.0118 mg/L, respectively. The post-dialysis iron concentrations in the plasma and ultrafiltrate were 1.110 and 0.0020 mg/L, respectively. Therefore, when ferric pyrophosphate was infused into the plasma by dialysis, there was nearly a three fold increase in the concentration of serum iron, and plasma iron exceeded the iron binding capacity of plasma. However, there was no increase in the concentration of free iron or low molecular weight iron chelates in the ultrafiltrate of the plasma, suggesting that iron in ferric pyrophosphate binds to plasma proteins and is not present as free iron. This method may be used to screen the presence of free iron and low molecular weight iron chelates, when iron compounds are administered parenterally.

Changes in the concentration of free
calcium, magnesium, zinc and copper

Changes in the concentration of free calcium, magnesium, zinc and copper in the plasma following the administration of iron allow estimation of the binding of the ligand to these metal cations. The plasma concentrations of these ions may be determined by methods well-known to those skilled in the art. Some iron compounds bind albumin and other plasma proteins, rather than transferrin. An example of such a compound

in ferrous gluconate. This binding is neither tight, nor specific. There remains a potential for toxicity. To measure the binding of iron specifically to transferrin, (and not to other proteins such as albumin) and to thereby make the assay more specific, plasma containing a variety of proteins can be substituted by a physiological salt solution to which physiologic concentrations of the protein transferrin have been added.

Detection of free iron in vivo

Previous studies have demonstrated that free iron present in the body fluids can be quantitated by the bleomycin binding assay; such iron is referred to as "bleomycin detectable iron" (Banyai et al., 1998). Therefore animal or human trials of the candidate iron compounds can use bleomycin detectable iron as a measure of free iron generated in the body fluids when the compound is administered.

Iron administration via dialysate in hemodialysis and peritoneal dialysis

According to one embodiment of the invention, a monomeric iron compound is infused via the dialysate in patients with renal failure.

Dialysis patients are those patients undergoing hemodialysis or peritoneal dialysis for renal failure. Long-term dialysis therapy for treatment of end stage renal failure is referred to as "maintenance dialysis". Patients on maintenance hemodialysis have been estimated to lose about 2 to 3 grams of iron per year, corresponding to approximately 6 ml per day (2 liters per year) blood loss from all sources (Eschbach et al., 1977). These patients generally receive hemodialysis three times per week.

A dialysis solution (dialysate) for hemodialysis can be generated using a variety of different methods and different dialysis machines. It is the purpose of the present invention to add the monomeric iron compound to the dialysate, regardless of the design of the dialysis machine or the manufacturing process for the dialysate. The various methods of generating the dialysate are summarized as follows:

1. Tank system: dialysate is mixed either from salts or liquid concentrates and water.

2. Continuos proportioning system as a central dialysate supply system.

3. Feedback controlled proportioning based on dialysate conductivity.

4. On-site production of dialysate from solid concentrate(s) and water.

Most dialysis machines produce dialysate continuously by mixing liquid or solid concentrates with water. There are two types of dialysates - acetate based and bicarbonate-based. Acetate dialysate is often prepared from a 35-fold concentrated solution. Bicarbonate dialysate must be produced form at least two concentrates. A first concentrate contains sodium bicarbonate; a second concentrate contains calcium and magnesium salts. The other constituents of the bicarbonate-based dialysate can be put in either concentrate. The most common system uses a 1-molar sodium bicarbonate concentrate and a so called "acid-concentrate" that contains sodium, potassium, calcium, and magnesium chloride as well as some acetic acid to adjust dialysate pH to about 7.3. Acid concentrates are diluted about 35-45 fold in the preparation of the dialysate. A system for continuous production of a saturated bicarbonate concentrate that is subsequently diluted with water to produce dialysate utilizes a cartridge filled with bicarbonate powder. Water percolates through the cartridge and the liquid exiting this cartridge at the bottom is a saturated bicarbonate solution. Alternative technical solutions using the same principle utilize bags instead of cartridges. The percolation principle can be applied to other salts including the iron compounds of this invention.

The compatibility of a monomeric iron compound with a dialysate or dialysate concentrate can be easily tested by determining solubility and stability of the compound when the two are mixed together, using standard laboratory techniques. The molecular weight of the monomeric iron

compound should be small enough to allow efficient diffusion across the dialysis membranes during hemodialysis and peritoneal dialysis. Fe (III) pyrophosphate, Fe (III) hydroxamates and Fe (III) hydroxypyridinones satisfy this requirement.

A specific example of a hemodialysis system is the Fresenius system. In the Fresenius system, the ratio of acid:bicarbonate:water:total is 1:1.23:32.77:35. Therefore, one part of the concentrated bicarbonate solution is mixed with 27.5 parts of the other (acid + water), to make the final dialysate. In order to make the bicarbonate concentrate, purified water is pumped from the purified water source into a large tank. Fresenius supplies sodium bicarbonate powder packaged in plastic bags and the contents of each bag are mixed with purified water in the tank, to make 25 gallons (94.6 liters) of bicarbonate solution. After thoroughly mixing with a stirrer, the concentrated solution is run into plastic receptacles. The concentrate is prepared within 24 hours of its use.

According to one preferred embodiment of the invention, the monomeric iron compound administered via dialysate is ferric pyrophosphate ("FePyP"). Ferric pyrophosphate ($Fe_4P_6O_{21}$) has a molecular weight of 745.2. It is a nonahydrate with yellowish-green crystals. It has been used as a catalyst, in fireproofing synthetic fibers and in corrosion preventing pigments. Ferric pyrophosphate is freely soluble in the bicarbonate concentrate. It may be added in a dry or solution form to the dialysis concentrate. For a dialysate iron concentration of 4 µg/dl or FePyP concentration of 40 µg/dl, it can be calculated that bicarbonate concentrate should have a ferric pyrophosphate concentration of 40 x 27.5 = 1100 µg/dl, or 11 mg/liter. Therefore, 1040 mg of ferric pyrophosphate added to 94.6 liter (25 gallons) of bicarbonate concentrate will generate a dialysate with an iron concentration of 4 µg/dl.

Table 3. Bicarbonate concentrates with a defined iron concentration achieved by addition of FePyP.

Required Conc. of Fe in dialysate	Estimated Conc. of FePyP in dialysate	Estimated Amount of FePyP in concentrate
2 µg/dl	20 µg/dl	5.5. mg/L
4µg/dl	40 µg/dl	11 mg/L
8 µg/dl	80 µg/dl	22 mg/L
12 µg/dl	120 µg/dl	33 mg/L

Dialysate iron concentration can be increased by adding different amounts of FePyP to the bicarbonate concentrate (Table 3). Ferric pyrophosphate may be added to the dialysate concentrate either in its crystalline form or as an aqueous solution.

As shown in Example I herein below, plasma (3.5 liters) was dialyzed in vitro using an F-80 dialyzer with the plasma flow rate set at 300 ml/min. and the dialysate flow rate 800 ml/min. Ferric pyrophosphate (420 mg) was added to 20 liters of bicarbonate concentrate and intermittently stirred for one hour prior to the dialysis. This was a clear solution with a light greenish yellow tinge. The final dialysate was a clear, colorless solution, with 5µg/dl iron content, as measured by a calorimetric assay. Physiological saline solution was added to the plasma every minute at regular intervals to compensate for obligate ultrafiltration, and to keep the plasma volume constant. Serum Fe and TIBC were measured at frequent intervals. There was a progressive increase in serum iron concentration (A), and transferrin saturation (B), as shown in Figure 1.

In a separate experiment, in vitro dialysis was performed using three different concentrations of ferric pyrophosphate in the dialysate. Under otherwise identical experimental conditions, the increment in transferrin saturation was dependent on the dialysate iron concentration (Figure 2).

Dialysis is defined as the movement of solute and water through a semipermeable membrane (the dialyzer) which separates the patient's blood from a cleansing solution (the dialysate). Four transport processes may occur simultaneously during dialysis:

1. Diffusive transport is the movement of solutes across the membrane, and is dependent on the concentration gradient between plasma water and dialysate;

2. Convective transport is the bulk flow of solute through the dialyzer in the direction of hydrostatic pressure difference;

3. Osmosis is the passage of solvent (water) across the membrane in the direction of the osmotic concentration gradient; and

4. Ultrafiltration is the movement of solute free water along the hydrostatic pressure gradient across the membrane.

The patient's plasma tends to equilibrate with the dialysate solution over time. The composition of the dialysate permits one to remove, balance or even infuse solutes from and into the patient. The electrochemical concentration gradient is the driving force that allows the passive diffusion and equilibration between the dialysate and the patient's blood compartment. The process of dialysis can be accomplished by using an artificial kidney (hemodialysis and hemofiltration) or patient's abdomen (peritoneal dialysis).

In an artificial kidney, a synthetic or semi-synthetic semipermeable membrane made of either cellulose acetate, cupraphane, polyacrylonitrile, polymethyl methacrylate, or polysulfone, is used. A constant flow of blood on one side of the membrane and dialysate on the other allows removal of waste products. An artificial kidney can be used to perform hemodialysis, during which diffusion is the major mechanism for solute removal. On the other hand, hemofiltration (also called hemodiafiltration and diafiltration)

relies on ultrafiltration and convective transport rather than diffusion to move solutes across a high porosity semipermeable membrane.

For the purposes of this invention, the term hemodialysis is used to include all dialysis techniques (e.g. hemofiltration) that require an extracorporeal blood circuit and an artificial membrane.

On the other hand, peritoneal dialysis uses patient's peritoneal membrane to exchange solutes and fluid with the blood compartment. Therefore, peritoneal dialysis is the treatment of uremia by the application of kinetic transport of water-soluble metabolites by the force of diffusion and the transport of water by the force of osmosis across the peritoneum. The peritoneum is the largest serous membrane of the body (approximately $2m^2$ in an adult). It lines the inside of the abdominal wall (parietal peritoneum) and the viscera (visceral peritoneum). The space between the parietal and visceral portions of the membrane is called the "peritoneal cavity". Aqueous solutions infused into the cavity (dialysate) contact the blood vascular space through the capillary network in the peritoneal membrane. The solution infused into the peritoneal cavity tends to equilibrate with plasma water over time and it is removed at the end of one exchange after partial or complete equilibration. The composition of the dialysate permits to remove, balance or even infuse solutes from and into the patient. The electrochemical concentration gradient is the driving force that allows the passive diffusion and equilibration between the dialysate and blood compartment.

Dialysis solutions (hemodialysis or peritoneal dialysis) of the present invention are characterized by an added monomeric iron compound, having a molecular weight less than about 12,000 daltons, preferably having a molecular weight of less than 5000 daltons. Optimally, the ferric compound should be 1) soluble in dialysis solutions in adequate concentrations; 2) efficiently transfer from the dialysate to the blood compartment; 3) bind to transferrin in the plasma and be available for use by tissue; 4) be well tolerated without any short or long term side effects; and 5) be economical.

Ferric pyrophosphate possess all the above characteristics and therefore is the preferred iron compound for use with the present invention, though other soluble ferric compounds may also be used, as discussed above.

Presently, hemodialysis machines utilize an automated proportioning system to mix salts in deionized water in specific proportions to generate the final dialysate solution. The dialysate concentrates are usually supplied by the manufacturer either as a solution ready to use or as a premixed powder that is added to purified water in large reservoirs. The concentrates are pumped into a chamber in the dialysis machine where they are mixed with purified water to make the final dialysate solution.

Generally, the ionic composition of the final dialysate solution for hemodialysis is as follows: Na^+ 132-145 mmol/L, K^+ 0-4.0 mmol/L, Cl^- 99-112 mmol/L, Ca^{++} 1.0 - 2.0 mmol/L, Mg^{+2} 0.25-0.75 mmol/L, glucose 0-5.5 mmol/L. The correction of metabolic acidosis is one of the fundamental goals of dialysis. In dialysis, the process of H^+ removal from the blood is mainly achieved by the flux of alkaline equivalents from the dialysate into the blood, thereby replacing physiological buffers normally utilized in the chemical process of buffering. In dialysis practice, base transfer across the dialysis membrane is achieved by using acetate or bicarbonate containing dialysate. In bicarbonate dialysis the dialysate contains 27-35 mmol/L of bicarbonate and 2.5-10 mmol/L of acetate. On the other hand, in acetate dialysis the dialysate is devoid of bicarbonate and contains 31-45 mmol/L of acetate. Ferric pyrophosphate in particular is compatible with both acetate and bicarbonate based hemodialysis solutions.

The peritoneal dialysis fluid usually contains Na^+ 132-135 mmol/L, K^+ 0-3 mmol/L, Ca^{++} 1.25-1.75 mmol/L, Mg^{++} 0.25-0.75 mmol/L, Cl^- 95-107.5 mmol/L, acetate 35 mmol/L or lactate 35-40 mmol/l, and glucose 1.5-4.25 gm/dL. Ferric pyrophosphate in particular is soluble and compatible with peritoneal dialysis solutions.

In accordance with the present invention, a suitable monomeric iron compound, e.g., ferric pyrophosphate, is either added directly to peritoneal

dialysis solutions, or to the concentrate for hemodialysis. In case of hemodialysis, since the concentrates are diluted several fold in the machine by admixture with water, the compound has to be added at a proportionally higher concentration in the concentrate.

Preferably, 2 to 25µg of the ferric iron (e.g., as ferric pyrophosphate) per deciliter of the hemodialysis solution is used for hemodialysis. Accordingly, 4 to 50 milligrams of iron are infused into the patient during a two to five hour hemodialysis session. Currently, hemodialysis patients number 230-250,000 in the United States and about one million worldwide. The majority of these patients require erythropoietin therapy to maintain hemoglobin in the target range of 10-12 gm/dL. Although, all patients on dialysis treated with erythropoietin are prescribed oral iron therapy, only 45% maintain transferrin saturation levels above 20 percent with oral iron therapy (Ifudu et al., 1996). It has been documented that at least one-half of the hemodialysis population requires intravenous iron to maintain iron balance (Sepandj et al., 1996). Even though dialysate iron therapy is potentially useful for all hemodialysis patients, those requiring intravenous iron are more likely to benefit.

To evaluate whether dialysate iron therapy is more economical than the conventional therapies, a comparative cost analysis for one patient year of hemodialysis was performed. It is estimated that a maximum of one gram of ferric pyrophosphate may need to be added to 20 liters of bicarbonate concentrate which is utilized during a single dialysis procedure. A total of 156 grams of ferric pyrophosphate will be added to the dialysate per patient year. The cost of FePyP is $25.00 per kg (Mallinckrodt Baker, Inc., Chesterfield, Missouri), and therefore, the annual cost of FePyP is estimated to be approximately $5.00 per patient year. It is evident that dialysate iron therapy is more economical than intravenous iron.

As shown in Example 2 herein below, the efficacy and safety of ferric pyrophosphate added to the dialysate is established. Uremic patients on chronic hemodialysis, receiving regular maintenance intravenous iron

were randomized into two groups. One cohort was selected to receive dialysate iron therapy, accomplished by adding soluble ferric pyrophosphate to the dialysate. The other cohort was continued on regular maintenance intravenous iron dextran. At baseline, there were no significant differences in the two groups as regards demographics, comorbid conditions (hypertension/diabetes), nutritional parameters (body weight, albumin, lipids), iron parameters, requirements for erythropoietin or intravenous iron dextran. In this dose-finding study, after six months of observation, the only significant difference between the two groups was a decline in intravenous iron requirement in the dialysate iron group (P=0.002). No adverse effects related to dialysate iron were identified.

In conclusion, addition of iron to the dialysate as ferric pyrophosphate, is a safe and effective method of iron administration to hemodialysis patients. Dialysate iron therapy is able to maintain iron balance in the majority of hemodialysis patients without a need for oral or intravenous iron supplementation. In a minority of patients receiving dialysate iron therapy, the requirement for intravenous iron is significantly reduced but not completely eliminated.

In view of the above, the present invention provides pharmaceutical composition of a soluble, noncolloidal ferric compound that can be added to dialysis solutions to meet the iron supplementation or therapeutic needs of dialysis patients. However, some dialysis patients may still need oral or intravenous iron supplements.

Intravenous iron administration

According to another embodiment of the invention, monomeric iron may be delivered intravenously, such as by continuous infusion.

Transferrin is responsible for conveying iron between sites of iron storage and utilization. It has a molecular weight of 77,000 and contains two metal-binding sites per molecule and also binds one bicarbonate anion per metal atom. The metal-binding sites are equivalent in their affinity for

iron but apparently not identical in biological activity. The rate at which an iron is donated to transferrin by an iron compound is highly dependent on the nature of the iron compound.

Free iron should not accumulate in the circulating blood, since it is toxic. Iron bound loosely to plasma proteins other than transferrin is also toxic. Therefore, iron in plasma and other body fluids should not exceed the iron binding capacity of transferrin; over-saturation of transferrin should be avoided. The rate of infusion of the monomeric iron compound should thus be slow enough to avoid transferrin over-saturation.

For intravenous infusion of monomeric iron compound according to the present invention, a stable access to the circulation is desirable, so that the total amount of iron needed by the patient can be infused over a period of hours to days. The following are non-limiting choices for intravenous access that may be used for slow intravenous infusion of monomeric iron compound according to the practice of the present invention:

(1) The iron compound may be administered by ordinary peripheral or central venous cannulas in hospitalized patients or patients visiting an outpatient clinic.

(2) The iron compound may be administered by an infusion pump such as the type presently used for home infusion of dobutamine or insulin.

(3) The iron compound may be administered by an indwelling catheter that may or may not be tunneled in the subcutaneous tissue. The catheter may comprise, for example, a Quinton, Hickman, or Groshong catheter as used for chemotherapy in cancer patients and for hemodialysis in end-stage kidney failure patients.

The iron compound may also be administered by other means of dialysis access such as an arteriovenous shunt; the shunt is accessed by insertion of needles for the purposes of dialysis. The monomeric iron compound may be infused into the extra-corporeal circuit directly as described later in this application.

The monomeric compound is administered in the form of a sterile solution when administered by the intravenous, subcutaneous, intramuscular or intra-peritoneal route. This is in contrast to administration via a hemodialysis solution, which must be clean but not necessarily sterile. The monomeric iron compound, as exemplified by ferric pyrophosphate, may be freely soluble in warm water and compatible in any physiologically acceptable aqueous solution such as physiological salt solution (saline, or Ringer's lactate) or isotonic dextrose solution. Such solutions can be supplied as pre-mixed, ready to use formulations or formulated easily in a hospital pharmacy, outpatient pharmacy, or at a patients bedside by mixing the said iron compound, in a solid or liquid phase to an appropriate diluting fluid such as dextrose or salt solution. When a concentrated solution of the iron compound is infused by a syringe pump at a very slow infusion rate the solvent may be a sterile solution containing salt, dextrose or just pure water. For parenteral administration of the monomeric iron compound, other than via the dialysate, water solubility is not a prerequisite. Hence, the monomeric iron compound can be infused even in non-aqueous solvents or suspension fluids, as long as the suspension, colloid or solution is stable and suitable for parenteral administration.

The rate of iron compound infusion should be such that transferrin saturation is not exceeded. It may be advisable to restrict the infusion rate whereby the percentage saturation of plasma iron binding capacity (transferrin saturation) is 80% or less.

In patients where long-term intravenous access is available, infusion of the monomeric iron compound can continue for days to weeks. Similarly, in outpatients, continuous or intermittent infusions may be given for days to weeks if a stable access is available, such as an indwelling catheter for chemotherapy in cancer patients. In such cases, the monomeric iron compound may be administered at a rate that equals the uptake and utilization of iron by tissues, such as the erythron. However, when it is necessary to restrict the infusion to a few hours, the efficiency of

administration can be improved by a short bolus infusion to raise transferrin saturation (TSAT) from a baseline level to about 80% in a few minutes, followed by infusion at a slower rate to maintain transferrin saturation at this high level for the duration of the infusion.

The amount of iron compound required to raise the transferrin saturation from baseline to peak can be calculated. In a patient weighing 100 kg, an estimated plasma volume of 4000 ml, plasma iron binding capacity of 250 mcg/dl or 2.5 mg/liter, the total iron binding capacity of plasma is about 10 mg. Since the extravascular compartment contains nearly the same amount of transferrin as the plasma compartment, the total iron binding capacity of transferrin present in plasma and interstitial compartment is about 20 mg iron. If the baseline transferrin saturation is 15% and iron administration is needed to raise the target transferrin saturation to 75%, a 60% increase in TSAT is desired. A 60% increase in total iron binding capacity of 20 mg equals 20 x 0.6 mg or 12 mg iron. Therefore, the patient would need 12 mg iron, as the said iron compound, to raise his transferrin saturation from 15% to 75% over a short period of time.

The maintenance rate for iron infusion is a function of the rate of tissue iron uptake. The rate of iron uptake by the tissues may be measured according to the procedure set forth in Example 6, wherein ferric pyrophosphate was infused in a patient with kidney failure receiving hemodialysis via the dialysate. Blood samples are taken before starting dialysis, at the end of dialysis and for an hour after dialysis had been completed. The decline in the serum iron concentration and transferrin saturation (TSAT) is determined at these time points. Based on the patients estimated plasma volume, plasma iron binding capacity, and the measured decline in transferrin saturation over one hour, the amount of iron (delivered as ferric pyrophosphate) taken up by the target tissues per hour is determined.

Therefore, the rate of exit of the monomeric iron compound from circulation, and therefore the rate at which the compound should be infused to maintain the desired transferrin saturation, can be estimated by measuring the rate of decline of serum iron levels after an infusion of the iron compound. As an example, a hypothetical patient has a measured transferrin saturation of 75% after infusion of 12 mg iron and the transferrin saturation declines to 50%, 1 hour after the infusion. A 25% decline in TSAT, when the total iron binding capacity of the patient is 20 mg, suggests a tissue-uptake of 5 mg iron over an hour. Therefore infusion of 5 mg iron (or about 50 mg ferric pyrophosphate) to such a patient would be expected to maintain TSAT at the desired sub-maximal level.

Subcutaneous iron administration

According to another embodiment of the invention, monomeric iron may be delivered subcutaneously, such as by a continuous release implant formulation containing the monomeric iron compound. Once in contact with the body fluids, a polymeric depot containing the iron compound utilizes two mechanisms for the release of iron. The first involves leaching of iron compound at or near the surface, essentially a diffusion/dissolution controlled event. The second involves bio-degradation of the polymer as it comes in contact with body fluids and release of iron compound from the interior of the depot. The depot formulation is selected to provide a consistent, gradual release profile. Slow release of iron from the depot will obviate the need for regular administration of oral iron in an iron deficient patient, and compliance with iron supplementation will be improved.

Transdermal iron administration

According to another embodiment of the invention, monomeric iron may be delivered transdermally. An appropriate monomeric iron-containing formulation may be applied to the skin of the patient as a paste, with or without an occlusive dressing. Alternatively, the iron compound may be

contained in an appropriate transdermal device or "patch". Iron is absorbed through the skin over prolonged periods of time, thereby obviating the need for oral iron.

Lipophilic substances have a tendency to sorb into the dense horny layer of the epidermis, that may provide a reservoir for slow release of medication. The horny layer is a considerable obstacle that even lipophilic substances can only penetrate slowly. Consequently the release of the drug such as an iron compound into the circulation is inhibited. The serum level of iron will be flat and low, or plateau-like, in contrast to the development of high serum levels, after an intravenous or oral bolus. The dense horny layer, with its reservoir capacity, therefore provides for a steady-state release of iron which avoids "roller coaster" drug delivery and its effects. The penetration rate, or flux, of the various candidate iron compounds can be tested by application of equivalent amounts of iron and measuring the serum iron levels.

It is known that compounds with one or more of the following properties penetrate poorly through the skin: high molecular weight macromolecules, compounds that are only water soluble and not lipid soluble, and water soluble electrolytes. Lipid-soluble iron compounds have the best chance of diffusing into the horny layer, especially if these compounds are non-polar and of moderately low molecular weight. Compounds that are both lipid and water-soluble partition most efficiently into the horny layer. Therefore, iron compounds of this invention may be screened and selected based on the properties that make compounds suitable for transdermal application.

Administration of anti-oxidants

According to another embodiment of the invention, anti-oxidants are administered as an adjunct to iron administration, in order to reduce oxidant stress and/or endothelial dysfunction.

The toxicity of simple iron salts or the polymeric iron complexes is secondary to liberation of free iron due to chemical interactions in plasma or infusion of free iron that is present in such preparations. Bleomycin-detectable free iron can be found in serum after the parenteral administration of colloidal iron (Banyai et al., 1998). The pathophysiologic effects are different depending on the molecular species of free iron, Fe(II) or Fe(III). Of these two common valences, Fe(II) is the most reactive form leading to the production of highly reactive hydroxyl radicals by the Fenton reaction, or alkoxyl and peroxyl radicals from the breakdown of lipid peroxides (reviewed by Gutteridge and Halliwell, 1990). That redox active iron is released by colloidal iron compounds in the circulation is further evidenced by the rise in plasma total peroxide and malondialdehyde concentrations within ten minutes following infusion of colloidal iron (Roob et al., 1998). In the anesthetized cat Fe(II) produces a hypotensive response which may be attributable to the oxidant stress. The free radical generation and lipid peroxidation catalyzed by free iron may also play a role in the pathogenesis of a variety of chronic diseases such as inflammation, ischemia, atherosclerosis, cancer, heart disease, and stroke. On the other hand, in the anesthetized cat, Fe(III) produces a smaller transient depressor response followed by a more sustained pressor response (Cox et al., 1965; Goldberg, 1958). The pressor response suggests that Fe(III) may induce endothelial dysfunction and interfere with the nitric oxide pathway.

It has been recently shown that pretreatment with antioxidant vitamin E and ascorbic acid blocks endothelial dysfunction and oxidant stress induced by homocysteine (Nappo et al., 1999).

According to the present invention, patients receiving monomeric iron compound may also receive one or more antioxidants to mitigate endothelial dysfunction and oxidant stress. According to one preferred embodiment, patients receive vitamin C, 1000 mg (range: 100 – 1,500 mg) and vitamin E, 800 IU (range: 100 to 1500 IU), prior to, at the time of, or

immediately after the administration of iron. Other antioxidants may be substituted.

The antioxidants(s) are preferably administered at the same time as the iron compound. Thus, a pharmaceutical composition that combines a therapeutic dose of iron (not daily recommended allowance as dietary supplement) with antioxidants is comprised of about 25 to 1500 mg of iron, in the form of a monomeric or polymeric iron complex, chelate or compound, about 100 to 10,000 units of vitamin E, and about 50 to 10,000 mg of vitamin C, or any other suitable antioxidants. This pharmaceutical composition may be suitable for oral or parenteral use based on the vehicles or solvents used, liquid or solid phase and sterility of the dosage form.

The following Examples demonstrate the preparation and utility of the present invention.

EXAMPLE I

IN VITRO STUDIES ON THE SOLUBILITY OF FERRIC PYROPHOSPHATE IN DIALYSIS SOLUTIONS

Ferric pyrophosphate ($Fe_4(P_2O_7)_3$, M.W. 745.2, CAS 10058-44-3) (hereinafter FePyP) is a greenish yellow, crystalline compound that is known to have a solubility of 50 mg per ml in warm water (Catalog no. P 626; Sigma Chemical Co., St. Louis, Missouri). Initially, a small amount of FePyP crystals were added to the acid (pH, 2.49) and basic (pH, 7.81) concentrates and a bicarbonate dialysate (pH, 7.15). FePyP dissolved readily in the bicarbonate dialysate and the bicarbonate concentrate, forming a yellow-orange solution. However, there was incomplete dissolution in the acid concentrate, where a precipitate was clearly visible. Since the concentrated bicarbonate solution is diluted several fold in the formation of the final dialysate, the concentration of FePyP in the bicarbonate concentrate should be appropriately higher than the desired dialysate concentration. Therefore, solubility of FePyP in the bicarbonate

concentrate was tested by adding variable amounts of FePyP and measuring the iron content of the mixture by a standard calorimetric method. The results are shown in Table 4:

Table 4. Concentration of iron in bicarbonate concentrate after the addition of ferric pyrophosphate

Amount of FePyP added	Expected Fe conc.	Measured Fe conc.
2 mg/ml	0.2 mg/ml or 20 mg/dl	20.250 mg/dl
5 mg/ml	0.5 mg/ml or 50 mg/dl	40.660 mg/dl
10 mg/ml	1.0 mg/ml or 100 mg/dl	94.500 mg/dl
20 mg/ml	2.0 mg/ml or 200 mg/dl	206.500 mg/dl

*note ~ 10% of FePyP is Fe

The measured and expected concentrations of iron were similar, showing that FePyP is highly soluble at the concentrations tested. In dialysis practice, dialysate with a specific concentration of FePyP can be generated using a bicarbonate concentrate containing a proportionately higher concentration of FePyP. Similar experiments were performed using the acetate concentrate for hemodialysis and ferric pyrophosphate was found to be soluble and compatible with acetate based dialysis solutions.

IN VITRO HEMODIALYSIS WITH DIALYSIS SOLUTIONS CONTAINING FERRIC PYROPHOSPHATE

In a second set of experiments, an *in vitro* dialysis of plasma, utilizing a conventional hemodialysis set up, was used to show that the addition of even small amounts of ferric pyrophosphate to a dialysate solution, results in significant transport of iron into the blood compartment

during dialysis. This occurs because the transferred iron avidly binds to transferrin in the plasma.

A. Methods

Plasma was obtained from a uremic patient undergoing plasma exchange therapy for Goodpastures syndrome. Citrated plasma was stored at -20°C in plastic bags. In three separate experiments, plasma was dialyzed against dialysates with different concentration of Fe, prepared by adding variable amounts of FePyP to the bicarbonate concentrate. Dialyzers with a polysulfone membrane (Fresenius, USA) were used. When the volume of plasma being dialyzed was less than 1000 ml, a small dialyzer (F-4, Fresenius) with small blood volume (65 ml) and surface area (0.8 sq. meter) was used at a plasma flow rate of 100 ml/min. With a larger volume of plasma, a F-80 dialyzer with a priming volume of 120 ml and a surface area of 1.8 sq. meter was used at a plasma flow rate of 300 ml/min. Heparin (500 units per hour) was infused to prevent clotting in the circuit. Serum was drawn at regular intervals during the experiment and serum iron (Fe), total iron binding capacity (TIBC) and transferrin saturation (Fe/TIBC x 100) were measured by a calorimetric assay. The obligate ultrafiltration of fluid during hemodialysis was compensated by an infusion of 0.9% saline. The iron parameters were corrected for net ultrafiltration by expressing the results as transferrin saturation.

B. Results

There was an increase in serum iron and transferrin saturation with time when iron was added to the dialysate (Figures 1 and 2). The increment in serum Fe and transferrin saturation was more as the concentration of iron in the dialysate was increased (Figure 2). There was a near doubling of transferrin saturation after two hours of dialysis with a dialysate iron concentration of 8 µg/dl (Figure 2).

Experimental parameters were chosen to mimic conditions that prevail in actual dialysis practice. Therefore, 3.5 liters of plasma (approximating the plasma volume in a 70 kg patient) was dialyzed against a dialysate with 5µg/dl Fe concentration. The results are shown in Figure 1.

The hourly increase in plasma iron concentration was 23, 23, 35 and 45 µg/dl, and the net increase in iron concentration was 140 µg/dl over the course of the experiment. Therefore, 5 mg iron (or ~50 mg FePyP) was infused into 3.5 liters of plasma, using a dialysate with 5 µg iron per dl.

In conclusion, ferric pyrophosphate can be added to the bicarbonate concentrate, to attain iron concentrations of 2-70 µg/dl in the final dialysate to meet various levels of Fe deficiency in patients. Hemodialysis with iron containing dialysate does result in transfer of iron to the blood compartment. In these *in vitro* experiments, maximum iron transfer cannot be obtained since transferrin is confined to a closed system. In vivo, the release of iron to the erythron in the bone marrow and to the tissues by transferrin, increases the total amount of iron that can enter the blood compartment. Thus, dialysate iron therapy is a safe and effective route of iron delivery to hemodialysis patients. In view of the above experiments, it is clear that hemodialysis utilizing a hemodialysis solution containing iron compounds such as ferric pyrophosphate, can be used to increase the amount of bioavailable iron in a mammal. The data demonstrates that the ferric pyrophosphate is soluble in hemodialysis solutions in adequate concentrations, efficiently transfers from the dialysate to the blood compartment, and binds to the transferrin in the plasma. This data demonstrates the utility of the present invention as a means for providing bioavailable iron in a mammal, but more specifically in dialysis patients requiring oral or parenteral iron supplementation.

EXAMPLE 2

ADMINISTRATION OF IRON TO HEMODIALYSIS PATIENT BY DIALYSIS, USING DIALYSIS SOLUTIONS CONTAINING SOLUBLE IRON: A PHASE I/II CLINICAL STUDY

A. Design of the Study

To determine a safe and effective dose of dialysate iron, a cohort of chronic hemodialysis patients were dialyzed with ferric pyrophosphate containing dialysate, while contemporaneous controls received regular doses of intravenous iron, in an open label, phase I/II clinical trial. All subjects in the study were receiving maintenance hemodialysis for end stage kidney failure, and requiring erythropoietin and intravenous iron to maintain hemoglobin in the 10-12 gm/dl range. After obtaining an informed consent, patients were enrolled and oral iron was discontinued. All patients received maintenance intravenous iron (50-100 mg every 1-2 weeks) during a 4 week long pre-treatment phase. The last two weeks of this pre-treatment period were used to establish the "Baseline" serum iron and hematological parameters. In the Treatment Phase, ten patients were dialyzed with iron containing dialysate (Dialysate-Fe group) for a period of 4 months. The concentration of iron in the dialysate was 2 µg/dl during the first 4 weeks, and was progressively increased every 4 weeks to 4, 8, and 12 µg/dl. Since adverse reactions were not experienced even with the maximum concentration, the trial utilizing 12 µg/dl dialysate iron was extended by an additional 2.5 months. Eleven control patients (IV-Fe Group) continued to receive up to 25-200 mg intravenous iron alone every 1-2 weeks, for the entire study period of 6.5 months.

The doses of intravenous iron dextran were adjusted based on the serum ferritin and transferrin saturation. Throughout the study, all patients were eligible to receive variable maintenance doses (0, 25, 50 or 100 mg) of supplemental IV iron dextran (INFeD®, Schein Pharmaceuticals Inc., NJ)

once a week during hemodialysis in order to maintain predialysis TSAT > 20% and ferritin > 100 µg/L.

If serum transferrin saturation were to exceed 50% or serum ferritin were to exceed 1500 µg/dl, the administration of intravenous or dialysate iron was discontinued. On the other hand, if any patient demonstrated evidence of a severe iron deficiency (i.e. transferrin saturation <15% or serum ferritin <50 µg/L), the subject was treated for iron deficiency by intravenous administration of 100-200 mg: iron with each dialysis session up to a total dose of 200-1000 mg at the discretion of the Inventor. Increased availability of iron to marrow cells may improve responsiveness to erythropoietin, thereby raising the hemoglobin and hematocrit. Hemoglobin and hematocrit were monitored every week, and in the event of improved erythropoiesis, the doses of erythropoietin were reduced by 10% every two weeks or as needed, to maintain a stable hemoglobin.

B. Choice of the control group

According to the National Kidney Foundation-Dialysis Outcomes Quality Initiative (NKF-DOQI) recommendations, most hemodialysis patients should be administered intravenous iron with every dialysis session or every 1-2 weeks (maintenance therapy). NKF-DOQI guidelines do not recommend continuation of oral iron supplements in chronic hemodialysis patients on maintenance intravenous iron. This was the basis why the control group was maintained on regular doses of intravenous iron, while oral iron was discontinued. This being the standard of care, the subjects on maintenance intravenous iron (IV-Fe Group) served as the control against the experimental group receiving dialysate iron therapy (Dialysate-Fe Group).

C. Study population

The study population was randomly selected from all patients undergoing maintenance hemodialysis at Clara Ford Dialysis unit. Patients who met the inclusion and exclusion criteria, as described below, were eligible for entry into the pre-treatment phase of the study only after the nature and purpose of the protocol had been explained to them and after they had voluntarily granted written informed consent to participate.

1. Inclusion Criteria Only patients meeting all of the following criteria were eligible for entry into the pre-treatment phase of the study:

- Patients who have voluntarily signed an informed consent;
- Patients aged 18 years or older;
- Patients with end stage renal disease undergoing maintenance hemodialysis, who are expected to remain on hemodialysis and be able to complete the study. Because of the relatively brief study period patients on cadaveric transplant list are not excluded.
- Patients, if female, must be either amenorrheic for a minimum of one year, or using an effective birth control method;
- Patients with a mild iron deficiency and therefore eligible for maintenance intravenous iron therapy in normal clinical practice.

2. Exclusion Criteria Patients exhibiting any of the following characteristics were excluded from entry into the study:

- Patients with severe iron deficiency defined as a transferrin saturation <15 % and/or serum ferritin <50 µg/L;
- Patients who are able to maintain adequate iron stores (transferrin saturation >25% and serum ferritin >200 µg/L) without parenteral iron therapy;
- Patients with a history of clinically significant allergic reaction to iron;
- Patients with malignancy or overt liver disease;
- Patients with a history of drug or alcohol abuse within the last 6 months;

- Patients considered to be incompetent to give an informed consent;
- Patients who are anticipated to be unable to complete the entire study (e.g. concurrent disease);
- Patients with hepatitis B, or HIV infection;
- Patients who are pregnant or breast feeding;
- Female patients who menstruate and are unwilling/unable to use a safe and effective birth control method to prevent pregnancy during the study period.

A random number generator was used to generate a list of 24 numbers. Odd and even numbers were assigned A or B designation respectively. A list of 23 patients was created based on the order in which consent was obtained for participation in the study. Patients were assigned to groups A or B based on their order in the list. Twenty-two patients entered the Treatment Phase. One patient in the dialysate iron group elected to withdraw from the study due to lack of interest on the first day of the treatment phase. The remaining twenty-one patients completed the study.

D. Dose Selection

1. Dose selection for Dialysate-Fe Group

Ferric pyrophosphate complexed with sodium citrate is soluble in aqueous solutions (ferric pyrophosphate soluble, Mallinckrodt Inc., St. Louis, Missouri). Soluble ferric pyrophosphate was dissolved in purified water and this solution was added to a freshly prepared bicarbonate concentrate solution. Dialysate solutions containing the desired concentration of soluble ferric pyrophosphate were generated by the addition of an appropriately higher concentration of the compound to the bicarbonate concentrate. A stable and clear dialysate solution containing up to 71 µg/dl iron as ferric pyrophosphate could be generated using this

method. Bicarbonate concentrate solutions were used within 24 hours of preparation to avoid bacterial growth. Based on previous *in vitro* hemodialysis studies using dialysate iron concentrations between about 2 and 70 µg/dl and taking patients' safety into consideration, in the present study, an initial dialysate iron concentration of 2 µg/dl was increased every 4 weeks to 4, 8 and then to a maximum of 12 µg/dl, which was then sustained for two additional months. When a relative iron deficiency was suspected bolus doses of 100-200 mg iron were administered intravenously with each dialysis, over 1-10 consecutive dialysis sessions.

2. Dose selection for IV-Fe Group

Based on the NKF-DOQI guidelines, patients in IV-Fe group were prescribed maintenance amount of intravenous iron from 25 to 100 mg/week. When a relative iron deficiency was suspected, bolus doses of 100-200 mg iron were administered intravenously with each dialysis, for up to 10 consecutive dialysis sessions.

E. Effectiveness and safely variables recorded

1. Effectiveness This variable was measured by:

• Monitoring the hemoglobin/hematocrit and iron parameters.

• Monitoring the dose of intravenous iron and erythropoietin in the two groups.

2. Safety Variables The following safety variables were measured and/or monitored frequently:

• Frequent monitoring of vital signs to detect any cardiovascular toxicity, respiratory toxicity or hypersensitivity reactions.

• Directed history and physical examination prior to any increment in the dialysate iron dose.

• Hemoglobin (for diagnosis of anemia)

• Iron parameters (for detection of iron deficiency or toxicity)

• Liver function tests (to detect hepatotoxicity)

• Nutritional parameters such as weight, albumin, cholesterol and triglycerides were measured to detect malnutrition.

• Serum electrolytes.

• Serum calcium and inorganic phosphorus: to detect any potential hypocalcemia or hyperphosphaemia secondary to ferric pyrophosphate administration.

F. Criteria for Effectiveness of Dialysate iron therapy

Experimental therapy will be considered effective, if the patients receiving iron in the dialysate, when compared with patients receiving maintenance intravenous iron;

• maintain hemoglobin level, without an increase in erythropoietin dose; and

• maintain adequate iron stores and did not develop iron deficiency despite a reduced need for intravenous iron. The three important tests of iron deficiency that were monitored in the study were TSAT (transferrin saturation), reticulocyte hemoglobin (Retic Hgb, a measure of the prevailing iron availability to the bone marrow) and serum ferritin (a measure of the tissue stores).

G. Concomitant therapy

• Oral iron was discontinued in both groups.

• Patients in the dialysate-Fe group received supplemental doses of intravenous iron when clinically indicated.

• Patients in both groups received blood transfusions when clinically indicated.

H. Statistical Methods and Analysis

Except for plotting of individual patient variables over time, the iron study data has been summarized prior to analysis. Descriptive analysis

was performed. Most of the analysis presented here, uses the data averaged over four or six/seven week intervals. A four week interval corresponds to the length of time each dose level was used during the dose escalation phase of the study. However, the final study interval used was six or seven weeks long, since the final data collection did not take place until twenty-six or twenty-seven weeks after the start of the intervention. (See Figures 3-15)

The baseline period, labeled month 0, included data for the four weeks immediately prior to the start of the intervention. (There was some data available for some or all the fifth week prior to the intervention, but data from this week is omitted from the formal data analysis.)

Weeks 1 to 4, when the dialysate dose of 2 µg/dl was employed, are labeled month "1", weeks 5 to 8 labeled month "2", weeks 9 to 12 labeled month "3", weeks 13 to 16 labeled month "4", weeks 17 to 20 labeled month "5", and weeks 21 to 26 (or 27) are labeled month "6".

Treatment doses, serum ferritin and transferrin saturation were plotted over time for each patient in each group. The proportion of patients who achieved optimal iron status in each group were computed as well as the average time required for this. Average serum ferritin and transferrin saturation levels were computed for each group at each time point.

The differences in mean serum ferritin and transferrin saturation levels were computed along with their 95% confidence intervals at each time point. The proportions of patients showing side effects, either serious or minor, were noted for each group at each time point.

Baseline demographic and nutritional status variables were analyzed from separate data sets. The nutritional parameters: weight, albumin, cholesterol and triglycerides were entered only once for each study month.

Data on instances of complications, medications and procedures was extracted from the Greenfield Health System database which contains routinely collected clinical information. For each variable the data was summarized as the count of days for a 4-week month, for which a

complication, medication administration or procedure was performed. If multiple instances occurred on a single day, this was counted as only one occurrence. Due to the infrequency of many of these variables, this data was summarized for the baseline month(0), for all 6 study months(1-6), and for the final observation month(6).

Data on pre- and post-hemodialysis weights and blood pressures, along with blood pressures recorded at times of complications during hemodialysis, were extracted from the Greenfield Health System database which contains routinely collected clinical information. The blood pressures were summarized by extracting the minimum and maximum for a session, since instances of hypotension and/or hypertension would be of interest.

I. Results of the study

1. Demographics and baseline of individual patients and comparability of treatment groups

Baseline characteristics of the 2 groups are shown in Table 5. None of the baseline differences were statistically significant.

Table 5: Characteristics of 21 patients included in the final analysis

Variable	Dialysate-Fe	IV Fe	p value
• *Demographics*			
Age (years)	53.5±14.3	58.1±15.5	0.489
Gender (Male)	6 (60%)	7 (64%)	0.788
Race (Black)	9 (90%)	11 (100%)	0.283
•*Co-morbid disease*			
Hypertension	10 (100%)	11 (100%)	1.000
Diabetes Mellitus	6 (60%)	7 (64%)	0.864
•*Nutritional Status*			
Albumin	3.8±0.45	3.8±0.38	0.870
Cholesterol	161.4±19.8	153.2±32.9	0.502
Triglycerides	156.8±75.5	143.7±73.8	0.693
Dry Weight	84.3±17.7	81.0±35.3	0.788

2. Hematological and Iron Parameters

During the study, the dose of erythropoietin and intravenous iron were adjusted and prescribed by the investigators so that hemoglobin/hematocrit and iron parameters (transferrin saturation and ferritin) would stay in the target range. In either group, there was no significant change in hemoglobin or TSAT/ferritin when parameters at the month 6 were compared with the baseline (Figures 4, 9 and 13). Furthermore, when the two groups were compared, there was no significant differences in hemoglobin (Figure 4), pre-dialysis serum iron, (Figure 6), TSAT (Figure 9), or ferritin (Figure 13) at months 0-6.

Testing for reticulocyte hemoglobin (Retic-Hgb) was not available during months 0-1, and consequently Retic-Hgb was measured only in months 2-6. At month 2, Retic-Hgb was 28.4 ± 0.9 pg in the Dialysate-Fe group vs. 27.0 ± 1.0 pg in the IV-Fe group ($p>0.1$). In both groups, Retic-Hgb did not change significantly during the course of the study (Figure 5).

a. Erythropoietin dose

The dose of erythropoietin did not change significantly during the study, in the two groups (Figure 14). Furthermore, there was no significant difference in the erythropoietin requirement between the two groups either at baseline or at any time during the study.

b. Dose of IV iron (Infed®)

During the pretreatment period (month 0), the average weekly dose of intravenous iron was 59.6 mg in the IV-Fe group and 68.7 mg in the Dialysate-Fe group (Figure 15). Despite no significant difference in hemoglobin, transferrin saturation, ferritin or erythropoietin dose between the two groups, the requirement for intravenous iron was significantly reduced with dialysate iron ($p \leq 0.002$ with 8-12 µg/dl dialysate iron).

The average weekly doses of intravenous iron were adjusted for baseline levels. In the Dialysate-Fe group, the average weekly dose of intravenous iron significantly declined from an average of 68.7 mg in month

0 to 8.9 mg in month 6 ($p<0.002$). The average weekly dose of intravenous iron in IV-Fe group did not change significantly from 68.7 mg in the baseline period to 56.2 mg in the 6th month ($p>0.7$). Furthermore, in month 6, only 2 out of the 10 patients receiving dialysate iron required additional intravenous iron supplements.

3. Transfer of iron from the dialysate to the blood compartment

The decrease in intravenous iron requirement in the Dialysate Fe group was accompanied by a dose dependent transfer of iron from dialysate to the blood compartment as reflected by the increment in serum iron with dialysis (Figure 7). With addition of iron to the dialysate, there was a dose dependent increase in post-dialysis TSAT (mean ± SD) to 31.7 ± 6.8% on 2 µg/dl, 37.0 ± 8.3% on 4 µg/dl, 54.7 ± 9.9% on 8 µg/dl and 71.75 ± 13.4% on 12 µg/dl (Figure 10). Hence the increment in TSAT and percentage change in TSAT during dialysis were dependent on the concentration of dialysate iron (Figures 11 and 12).

4. Total iron binding capacity

The baseline total iron binding capacity (TIBC, mean ± S.D.) was 222.3 ± 43.8 µg/dl in Dialysate-Fe group and 192.7 ± 48.1 µg/dl in IV-Fe group, and the difference between the two groups was not significant ($p>0.14$) (Figure 8). TIBC at 6 months, adjusted for the baseline values, was significantly higher in the Dialysate-Fe group ($p<0.05$). Circulating transferrin increases in the presence of iron deficiency. However, based on reticulocyte hemoglobin and serum iron parameters, there was no difference in the iron status between the two groups. Transferrin can be suppressed in patients with reticuloendothelial block and anemic of chronic disease. However, nutritional parameters, serum ferritin and reticulocyte hemoglobins in the two groups do not suggest that patients in the IV-Fe group were sicker or had a reticuloendothelial block in iron release.

Therefore, the reason for a difference in TIBC between the two groups towards the end of the study remains unclear.

5. Tissue stores of iron

Serum ferritin is a marker for the tissue stores of iron. To ensure adequate supply of iron to the bone marrow, the recommended target range for serum ferritin in the dialysis patients receiving erythropoietin therapy is 100 - 500 µg/L. The baseline serum ferritin was 154 ± 120 µg/L in Dialysate-Fe group and 261 ± 211 µg/L in the IV-Fe group (mean ± S.D.), and the difference between the two groups was not statistically significant (Figure 13). There was no significant change in serum ferritin, in either group, during the course of the study. The serum ferritin level in month 6 was 154 ± 120 µg/L in Dialysate-Fe group and 261 ± 211 µg/L in the IV-Fe group (mean ± S.D.), and the difference between the 2 groups was not statistically significant (Figure 13). These results demonstrate that infusion of iron with every dialysis session by the dialysate route does not lead to excessive tissue accumulation of iron or iron overload.

6. Safety Results

No adverse effects secondary to the use of dialysate iron therapy were identified. Specifically, monitoring of vital signs, physical symptoms or signs and laboratory parameters did not reveal any evidence of pulmonary, cardiovascular or liver toxicity. None of the patients receiving dialysate iron manifested any allergic or anaphylactic reactions. Dialysate iron did not have any significant effect on serum calcium or phosphate concentrations.

7. Summary and Conclusions

In maintenance hemodialysis patients, over a period of 6 months, dialysate iron therapy is:

(a) safe and does not lead to hypotension or anaphylaxis;

(b) maintains iron balance in approximately 80% of patients without supplemental oral or intravenous iron;

(c) the requirements for intravenous iron may be reduced by about 80%;

(d) maintains hemoglobin without an increase in erythropoietin requirement;

(e) does not lead to iron overload.

EXAMPLE 3

PERITONEAL DIALYSIS WITH SOLUTIONS CONTAINING FERRIC PYROPHOSPHATE FOR IRON SUPPLEMENTATION IN RABBITS

Peritoneal dialysis (PD) patients are less prone to iron deficiency than hemodialysis patients. However, PD patients lose blood through the gastrointestinal tract and from phlebotomy for laboratory tests. Furthermore, iron utilization is increased in dialysis patients treated with erythropoietin. Consequently, iron deficiency is common in PD patients. Iron supplementation in PD patients is commonly accomplished by the oral route, since intravenous access is not as readily available in PD patients. In fact, peripheral intravenous access may be impossible to obtain in some patients when the veins have been thrombosed by venesection or cannulation. In this situation, intravenous iron infusion would necessitate cannulation of a central vein. Both oral and intravenous routes of iron deficiency are associated with numerous side effects. Therefore, addition of iron compounds to peritoneal dialysis solutions merits investigation as an alternative means of iron delivery because of the ease of administration. This method would also be expected to provide a slow continuous and more physiological replacement of ongoing iron losses.

Intraperitoneal administration of iron has been tested in rats with disappointing results. Peritoneal dialysis with a dialysate solution containing 984 µg/dl iron (as colloidal iron dextran) failed to increase the serum iron concentration after 6 hours (Suzuki, *et al.*, 1995). Higher concentrations of iron dextran, though successful in increasing serum iron

concentration, are toxic to the peritoneum. Iron dextran induces an inflammatory response leading to peritoneal adhesions and fibrosis, and a brownish pigmentation of the peritoneal membrane from deposition of iron aggregates (Park *et al.*, 1997). Therefore, colloidal iron dextran is not suitable for administration by the intraperitoneal route. Other colloidal iron compounds are likely to have a similar toxic effect on the peritoneum. A soluble iron salt, ferric chloride, had been tested previously by the same group (Suzuki, *et al.,* 1994). In this study, despite a dialysate iron concentration of 400 µg/dl (as ferric chloride), there was no change in the serum iron concentration after 6 hours of peritoneal dialysis (Suzuki, *et al.*, 1994).

Results of a Phase I/II trial of iron delivery by the dialysate route in maintenance hemodialysis patients suggest that this is safe, effective and well tolerated. Therefore, addition of soluble ferric pyrophosphate to the peritoneal dialysis solutions was tested as a potential treatment for iron deficiency, in a rabbit model of acute peritoneal dialysis.

A. Materials and Methods

New Zealand white rabbits (n=10) on a standard rabbit diet containing 16 µg iron per kg and weighing 2.5-3.5 kg, were obtained. Control rabbits (n=3) continued to receive the standard diet. Seven rabbits were switched to an iron deficient (20-25 parts per million elemental iron) diet to produce a state of iron deficiency (iron-deficient group).

On day 1, blood was drawn from the central artery of the ear, using a 22g butterfly needle. Whole blood hemoglobin, serum iron and total iron binding capacity (TIBC) were estimated. A total of 10 ml blood was drawn from control rabbits and 20 ml from rabbits on an iron deficient diet. More blood was drawn from rabbits on the iron deficient diet to exacerbate iron deficiency. On days 7 and 14, another 8-10 ml blood was drawn from all ten rabbits for hemoglobin and iron studies.

Peritoneal dialysis was performed only in the iron deficient group. The volume of peritoneal dialysate per exchange was about 210 ml (70 ml/kg body weight) and the dialysis was performed only on days 14, 21, and 28.

B. Preparation of a Peritoneal dialysis solution containing ferric pyrophosphate

The dialysate was prepared by adding a sterile filtered ferric pyrophosphate solution to a 2 liter bag of peritoneal dialysis solution (4.25% Dianeal®). The iron concentration in the final dialysate was 500 µg/dl.

C. Procedures and Data Analysis

Rabbits were sedated using a subcutaneous injection of 2 mg/kg acepromazine and 0.2 mg/kg butorphanol, and restrained on a board in a supine position. Blood was drawn for hemoglobin and iron studies. The skin over the abdominal wall was shaved, disinfected with betadine and anesthetized by instillation of 1% lidocaine. An l8g angiocath was advanced into the peritoneal cavity for infusion of dialysis solution. After 210 ml dialysate had been infused from a 2 liter bag, infusion was stopped, the angiocath was removed and the rabbit was returned to its cage.

Blood samples were drawn for iron studies, 30 and 120 minutes after starting dialysis. After the 120 minute blood draw, the rabbit was sedated as described previously and restrained in a prone upright position. An 18g angiocath was reinserted into the peritoneal cavity and the dialysate was drained by gravity. After the dialysate had stopped draining, the angiocath was removed and the rabbit was returned to its cage.

Serum iron level was estimated by a calorimetric method, after separating iron from transferrin and then converting it into divalent iron. The total iron binding capacity (TIBC) was measured using the modified method of Goodwin.

The serum iron levels and transferrin saturation were compared at 0, 30 and 120 minutes using the Wilcoxon signed rank test. A P value of less than 0.05 was considered statistically significant. The study protocol was approved by the Institutional Review Board for the care of animal rights.

D. Results

A significant decrease in baseline serum iron and transferrin saturation was observed in rabbits that were fed an iron deficient diet, compared with the control group (Figures 16 and 18). The hatched rectangles in Figures 16-18 represent mean ± S.D values in the control group.

Iron deficient rabbits were dialyzed with a dialysis solution containing ferric pyrophosphate. Peritoneal exchanges were performed on study days 14, 21 and 28. Similar results were seen in all experiments. Results of the experimental dialysis performed on day 21 are described below.

During the course of peritoneal dialysis, a significant increase in serum Fe and transferrin saturation was evident at 30 minutes ($P<0.03$). Consequently, the mean serum iron and transferrin saturation increased into the normal range, in this group of iron deficient rabbits within 30 min. of starting dialysis. Peritoneal dialysis was continued for a total period of 2 hours. The significant increase in serum levels of iron and transferrin saturation was sustained for the duration of the experiment.

On day 28, after the final dialysis had been completed, all the animals were euthanized and specimens of the visceral and parietal peritoneum were obtained for histologic examination. No significant macroscopic or significant microscopic changes were observed and no significant iron deposition was detected by Prussian blue staining. Therefore, ferric pyrophosphate does not have any acute toxic effects on the peritoneal membrane.

E. Summary

The above is an example of (1) a novel formulation for iron supplementation in peritoneal dialysis; and (2) the first demonstration that addition of soluble iron salts to the peritoneal dialysate is a feasible method of iron delivery.

EXAMPLE 4

ADMINISTRATION OF SOLUBLE IRON BY PARENTERAL ROUTES

Dialysis involves diffuse transport of molecules across a semipermeable membrane. For a molecule that is present on both sides of the membrane, there is transport in both directions but the net transport occurs along the concentration gradient. Free plasma iron is highly toxic and therefore, almost all circulating iron is bound to proteins and plasma concentration of free iron is negligible. Consequently, during dialysis there is no transfer of iron from the blood to the dialysate compartment. In fact, when ferric pyrophosphate is added to the dialysate, there is a one way transfer of iron to the blood compartment during dialysis. This resembles parenteral delivery by routes such as intravenous, intramuscular, subcutaneous, or transdermal. Therefore it is possible to administer ferric pyrophosphate, and other iron compounds, parenterally by these routes, both in dialysis and non-dialysis patients.

In the clinical trial of ferric pyrophosphate in hemodialysis patients, the average increment in serum iron concentration during a 3-4 hour dialysis session was about 140 µg/dl. Assuming a plasma volume of 3.5 liters, it can be estimated that the increment in circulating iron bound to transferrin was about 5.25 mg per dialysis session. The extravascular space contains about as much transferrin as the intravascular space, and there is a free exchange of iron in between the two pools of transferrin. Therefore, it can be estimated that a total of about 10.5 mg iron (or about 105 mg ferric pyrophosphate) was transferred to the patient during a dialysis session. This indicates that in dialysis or non-dialysis patients, it

is possible to infuse a sterile solution of ferric pyrophosphate at a rate of about 40 mg per hour. Intermittent or continuous intravenous infusion may be administered if an intravenous access is available. In non-hemodialysis patients, intravenous access may be difficult, and it may be possible to deliver ferric pyrophosphate by subcutaneous implants, or by a transdermal delivery system.

In summary, ferric pyrophosphate, and other suitable iron compounds, may be delivered by the dialysate route in hemodialysis (Examples I and 2), peritoneal route in peritoneal dialysis patients (Example 3), or intravenous/subcutaneous/intramuscular/transdermal routes in dialysis or non-dialysis patients (Example 4).

EXAMPLE 5

REGULATION OF HEMATOLOGIC PARAMETERS IN DIALYSIS PATIENT BY MODIFICATION OF DIALYSIS SOLUTIONS

The results of the clinical study in Example 2, demonstrate a novel method of hematologic manipulation during dialysis by modification of dialysate solutions, as exemplified by the maintenance of hematological parameters in a narrow target range by regular delivery of iron by dialysis.

The oral or intravenous methods of iron delivery are often unable to maintain optimal iron balance in dialysis patients. With continued loss of iron and increased iron consumption during erythropoietin therapy, iron deficiency develops. As hemoglobin/hematocrit declines, the dose of erythropoietin is often increased and iron administered intravenously, to maintain hemoglobin/hematocrit in the target range. Consequently, hemoglobin/hematocrit rise and this phenomenon has been termed "hematocrit or hemoglobin cycling"

Administration of ferric pyrophosphate by the dialysate route during every dialysis session is able to maintain levels of iron, transferrin saturation (Figures 6 and 9) and hemoglobin (Figure 4) in a narrow target range. Therefore, dialysate delivery of ferric pyrophosphate abolishes

hematocrit cycling (Figure 4), by maintaining an optimal iron delivery to the erythron (Figure 5). This is also the first example of hematological manipulation by modification of dialysate.

EXAMPLE 6

DETERMINATION OF TISSUE IRON UPTAKE AND INFUSION RATE IN HEMODIALYSIS

The following example illustrates a method for measuring the rate of iron uptake by the tissues when an iron compound is infused. Ferric pyrophosphate was infused in a patient with kidney failure receiving hemodialysis via the dialysate, at a dialysate iron concentration of 12 mcg/dl. Blood samples were taken before starting dialysis, at the end of dialysis and for an hour after dialysis had been completed. There was a rapid decline in the serum iron concentration and transferrin saturation (TSAT) from 125 mcg/dl and 80% respectively at the end of dialysis to about 80 mcg/dl and 50% respectively, after one hour (Fig. 19). Based on the patient's estimated plasma volume, plasma iron binding capacity, and a 30% decline in transferrin saturation over one hour, it was estimated that about 3-5 mg of iron (delivered as ferric pyrophosphate) was taken up by the target tissues per hour. Therefore, suitable maintenance infusion rate for the patient would be 3-5 mg of iron, or 30-50 mg of ferric pyrophosphate, per hour.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims the invention may be practiced otherwise than as specifically described.

All references cited in this disclosure are incorporated herein by reference.

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I CLAIM:

1. A composition comprising a dialysate or dialysate concentrate suitable for hemodialysis or peritoneal dialysis and a monomeric iron compound comprising one or more iron atoms bound to one or more ligands, which iron compound:

(a) is able to donate a substantial portion of its iron content directly to the protein transferrin under physiological conditions;

(b) does not induce significant binding of the contained iron to proteins other than transferrin, or to other ligands found in body fluids, under physiological conditions;

(c) does not contain a ligand which significantly complexes with metal ions normally present in body fluids, under physiological conditions;

(d) does not release a clinically significant amount of free iron to body fluids, under physiological conditions;

(e) has a molecular weight of less than about 12,000 daltons;

(f) is water-soluble; and

(g) is other than ferric pyrophosphate.

2. The composition of claim 1 wherein the iron is Fe (III).

3. The composition of claim 2 wherein the log of the conditional stability constant of the iron compound is at least about 6 in a physiological electrolyte solution.

4. The composition of claim 2 wherein the ligand is a hydroxamate or a hydroxypyridinone.

5. The composition of claim 2 wherein the ligand is a natural or synthetic siderophore or siderophore derivative.

6. The composition of claim 2 further comprising one or more antioxidants.

7. The composition according to claim 2 which is a dialysate containing from about 1 to about 500 µg/dl of ferric iron.

8. The composition according to claim 7 which is a dialysate containing from about 1 to about 70 µg/dl of ferric iron.

9. The composition according to claim 2 which is a dialysate concentrate containing from about 0.3 to about 35 mg/L of ferric iron.

10. The composition according to claim 2 which is a dialysate generated for administration to the patient by flowing water or an electrolyte solution through iron present in the solid phase.

11. A method of iron administration to a dialysis patient comprising administering to the patient a hemodialysis solution or peritoneal dialysis solution containing an effective amount of a non-toxic, monomeric iron compound comprising one or more iron atoms bound to one or more ligands, which iron compound:

(a) is able to donate a substantial portion of its iron content directly to the protein transferrin under physiological conditions;

(b) does not induce significant binding of the contained iron to proteins other than transferrin, or to other ligands found in body fluids, under physiological conditions;

(c) does not contain a ligand which significantly complexes with metal ions normally present in body fluids, under physiological conditions;

(d) does not release a clinically significant amount of free iron to body fluids, under physiological conditions;

(e) has a molecular weight of less than about 12,000 daltons; and

(f) is water-soluble; and

(g) is other than ferric pyrophosphate;

wherein the iron compound is infused into the circulation of the patient during dialysis by diffusion from the dialysis solution across a semipermeable membrane.

12. The method of claim 11 wherein the iron is Fe (III).

13. The method of claim 12 wherein the log of the conditional stability constant of the iron compound is at least about 6 in a physiological electrolyte solution.

14. The method of claim 12 wherein the ligand is a hydroxamate or a hydroxypyridinone.

15. The method of claim 12 wherein the ligand is a natural or synthetic siderophore or siderophore derivative.

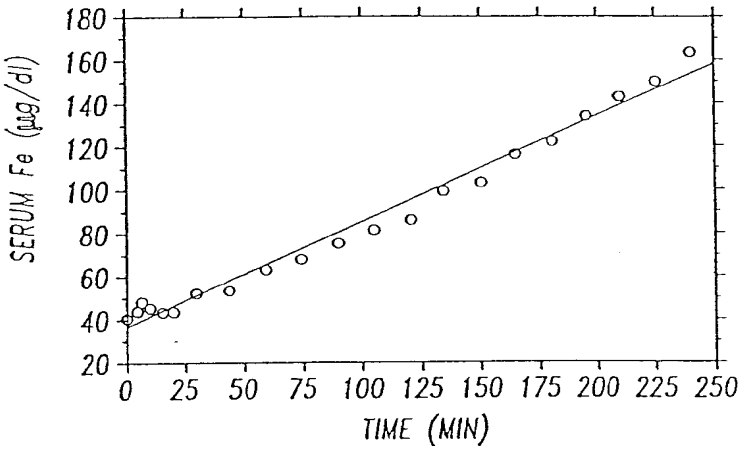
16. The method of claim 12 wherein the dialysis solution contains one or more antioxidants.

17. The method according to claim 16 wherein the antioxidant is selected from the group consisting of vitamin C and vitamin E, and mixtures thereof.

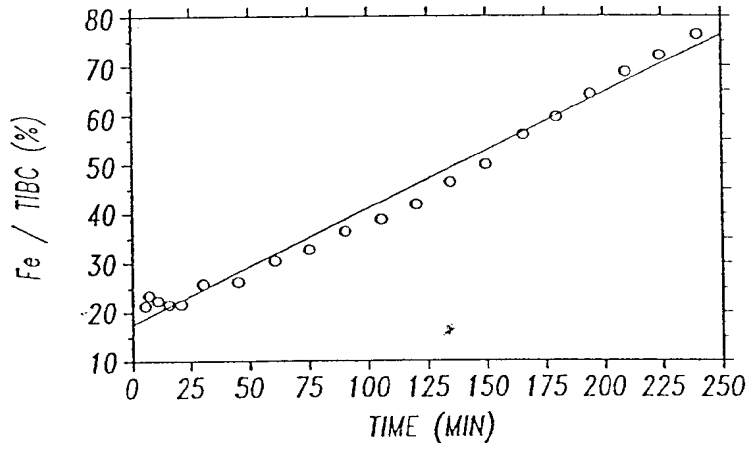
18. The method according to claim 12 wherein an antioxidant is administered to the patient by the parenteral or oral route, at a time proximal to the dialysis.

19. The method according to claim 12 which is a dialysate containing from about 1 to about 500 µg/dl of ferric iron.

20. The method according to claim 19 which is a dialysate containing from about 1 to about 70 µg/dl of ferric iron.



Fig–1A



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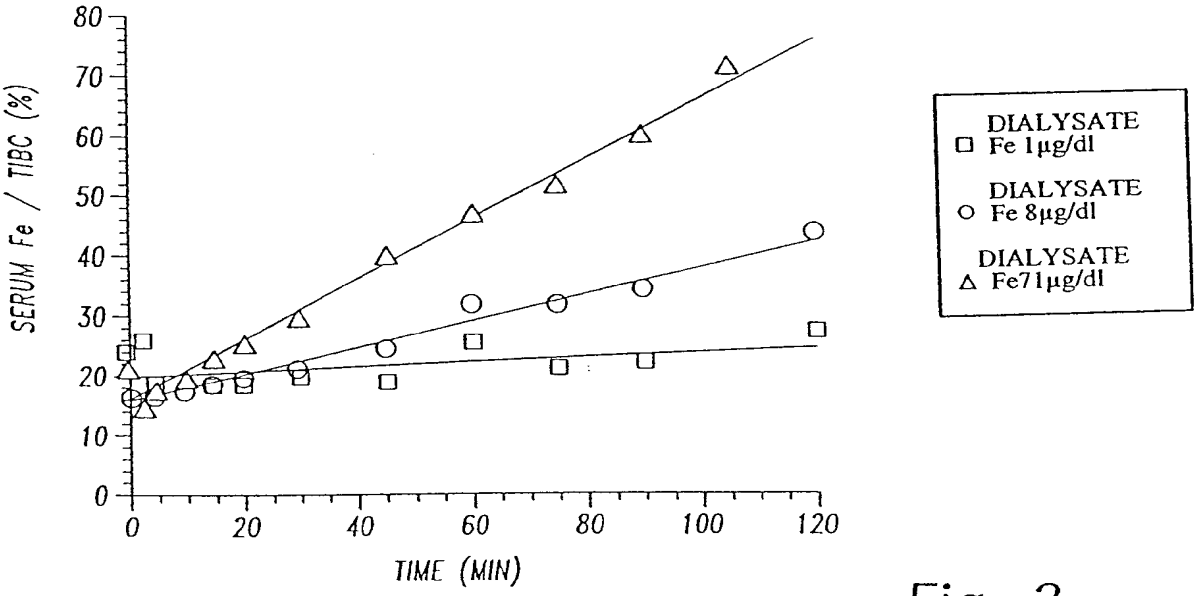


Fig–2

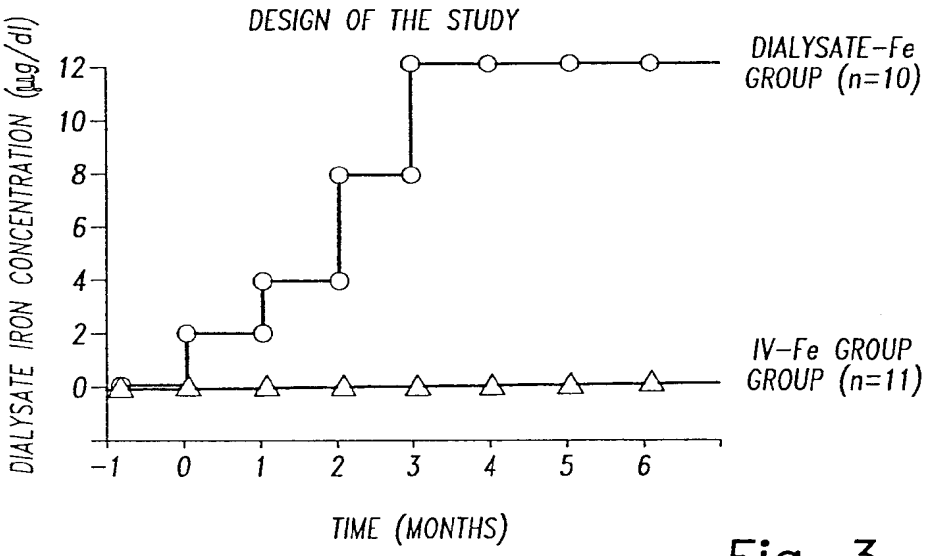


Fig-3

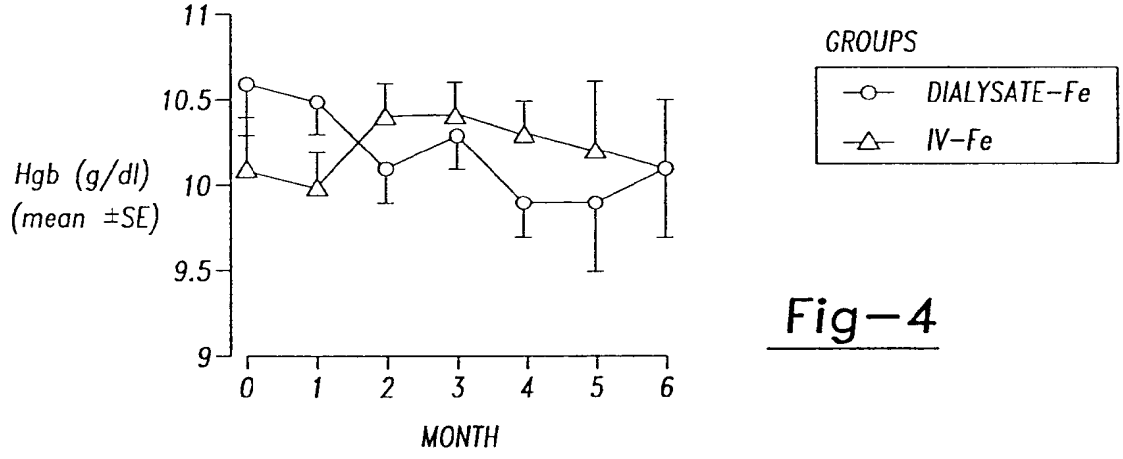


Fig-4

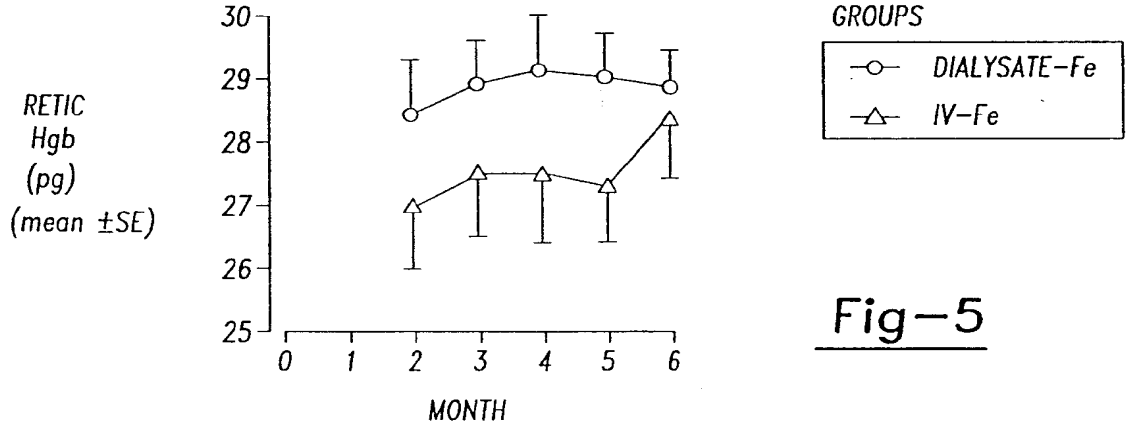


Fig-5

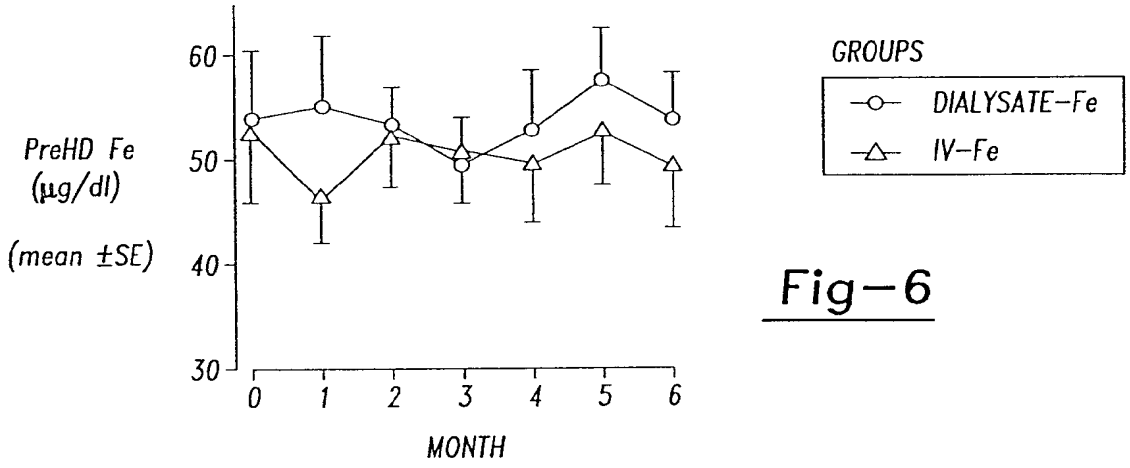


Fig-6

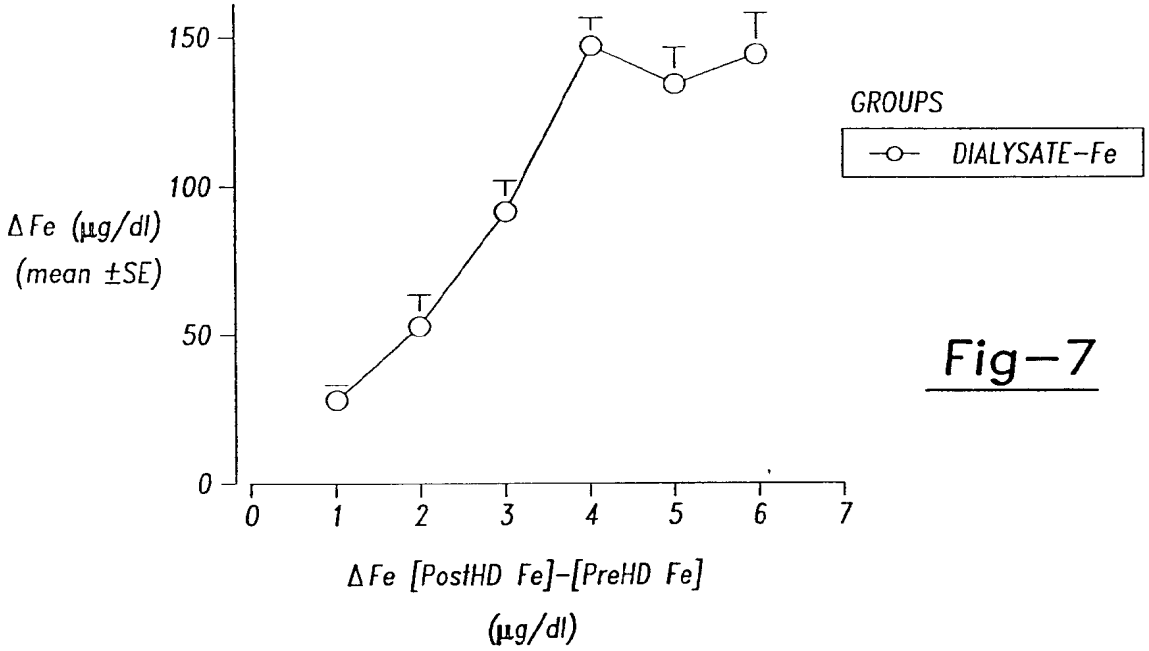


Fig-7

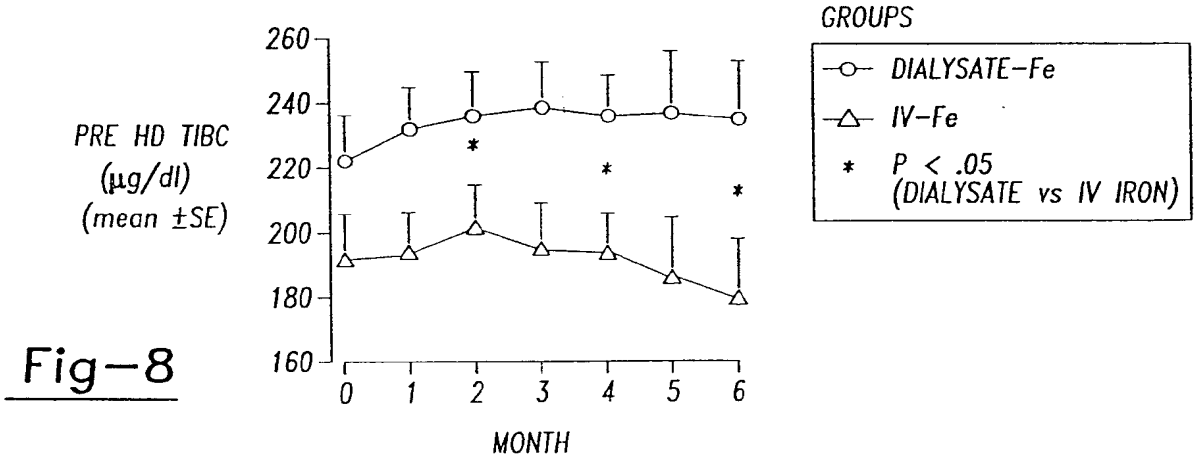


Fig-8

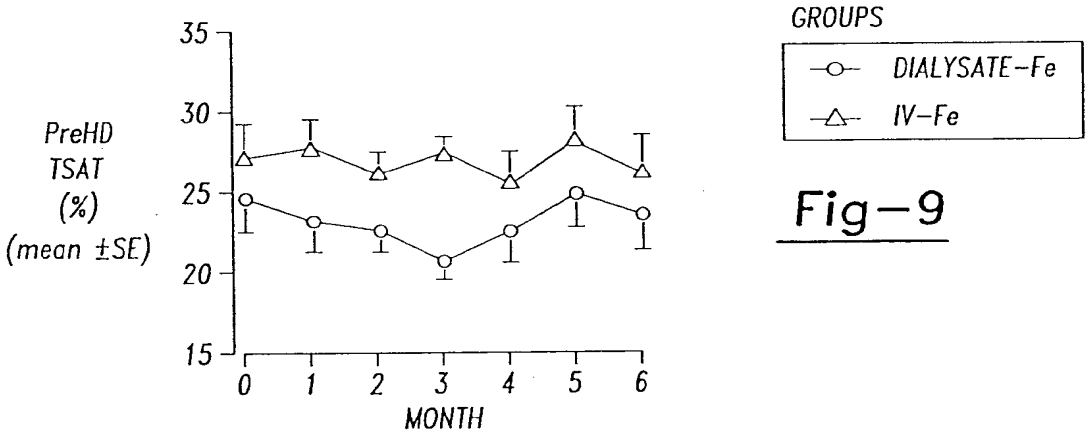


Fig-9

$$TSAT = \frac{SERUM\ Fe}{SERUM\ TIBC} X\ 100$$

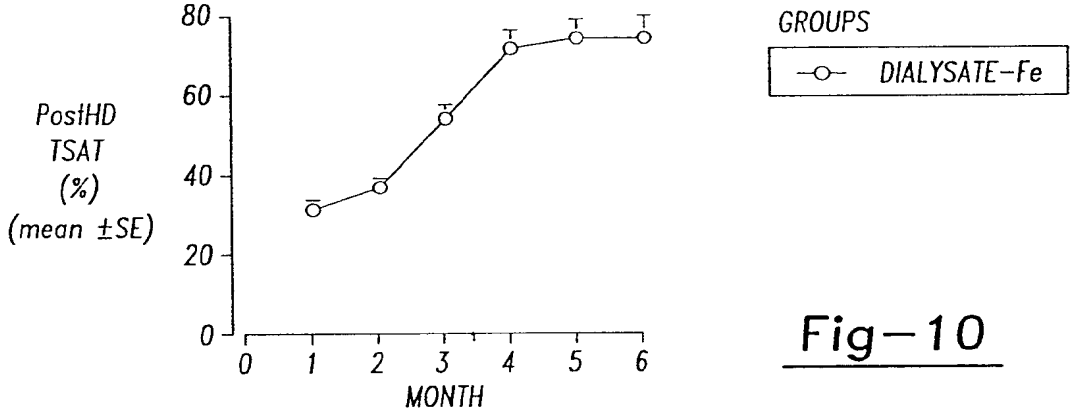


Fig-10

$$TSAT = \frac{SERUM\ Fe}{SERUM\ TIBC} X\ 100$$

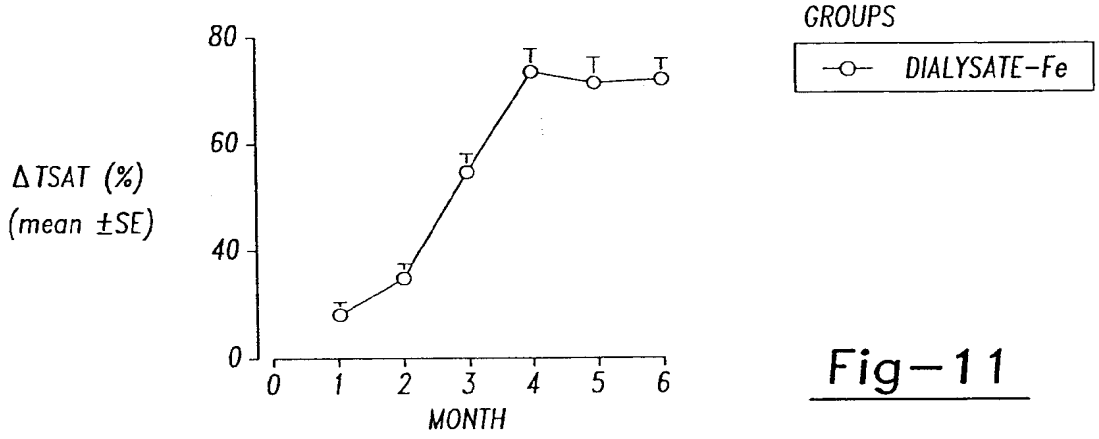


Fig-11

Δ TSAT (%)=[PostHD TSAT]-[PreHD TSAT]

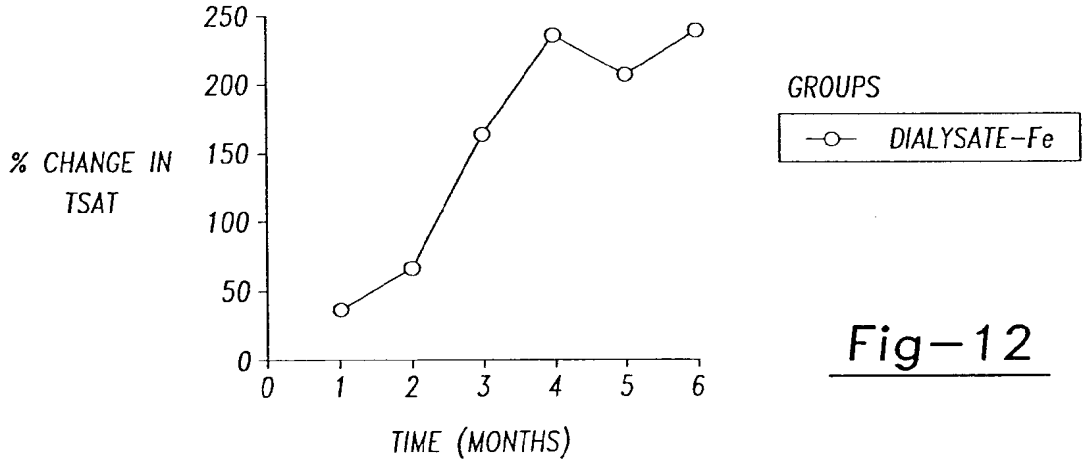


Fig-12

$$\%\text{CHANGE IN TSAT} = \frac{(\text{PostHD}) - (\text{PreHD})}{(\text{PreHD})} \times 100$$

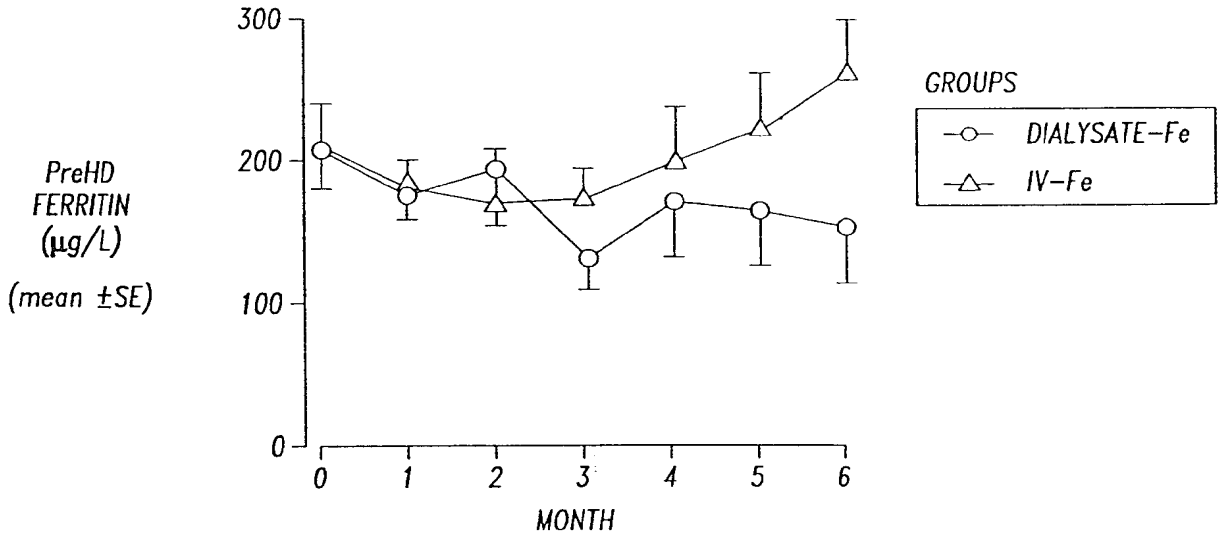


Fig-13

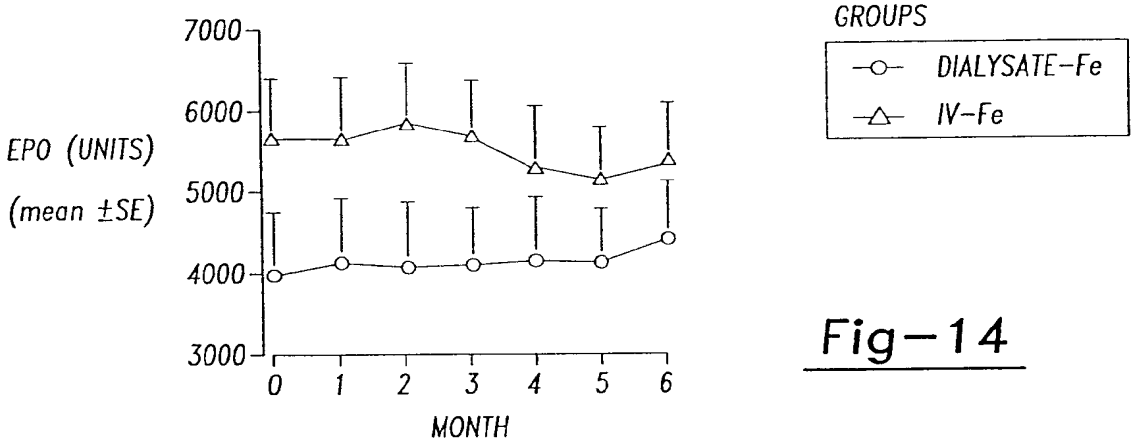


Fig-14

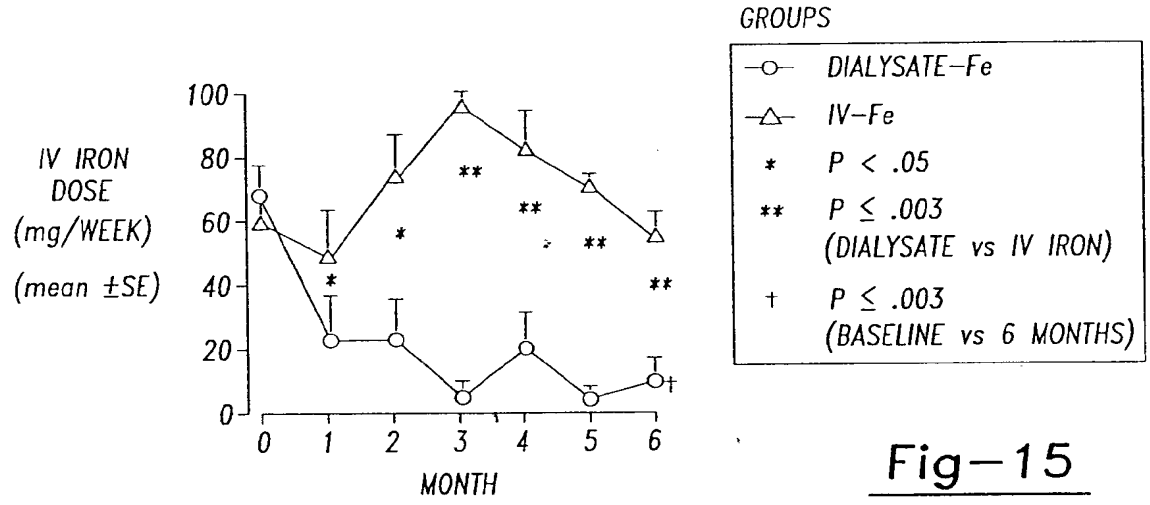


Fig-15

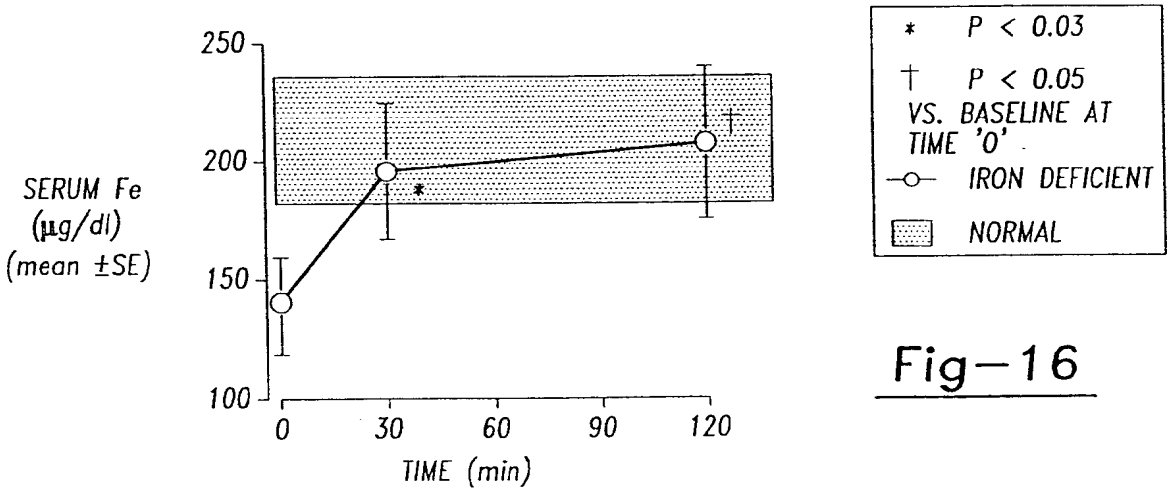
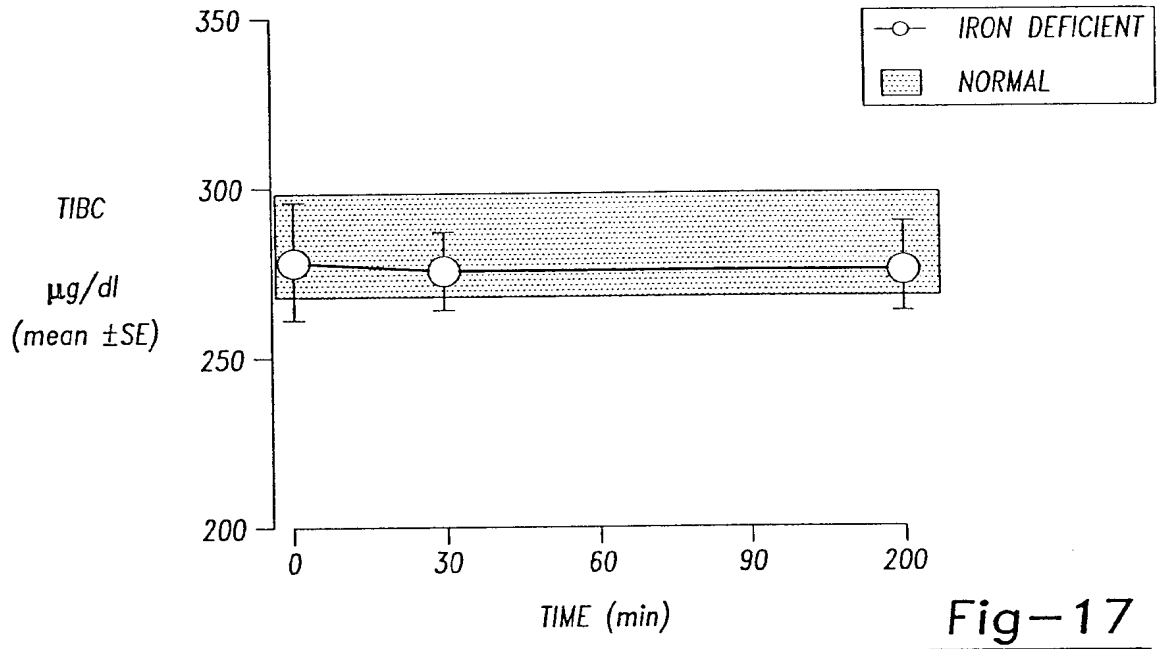
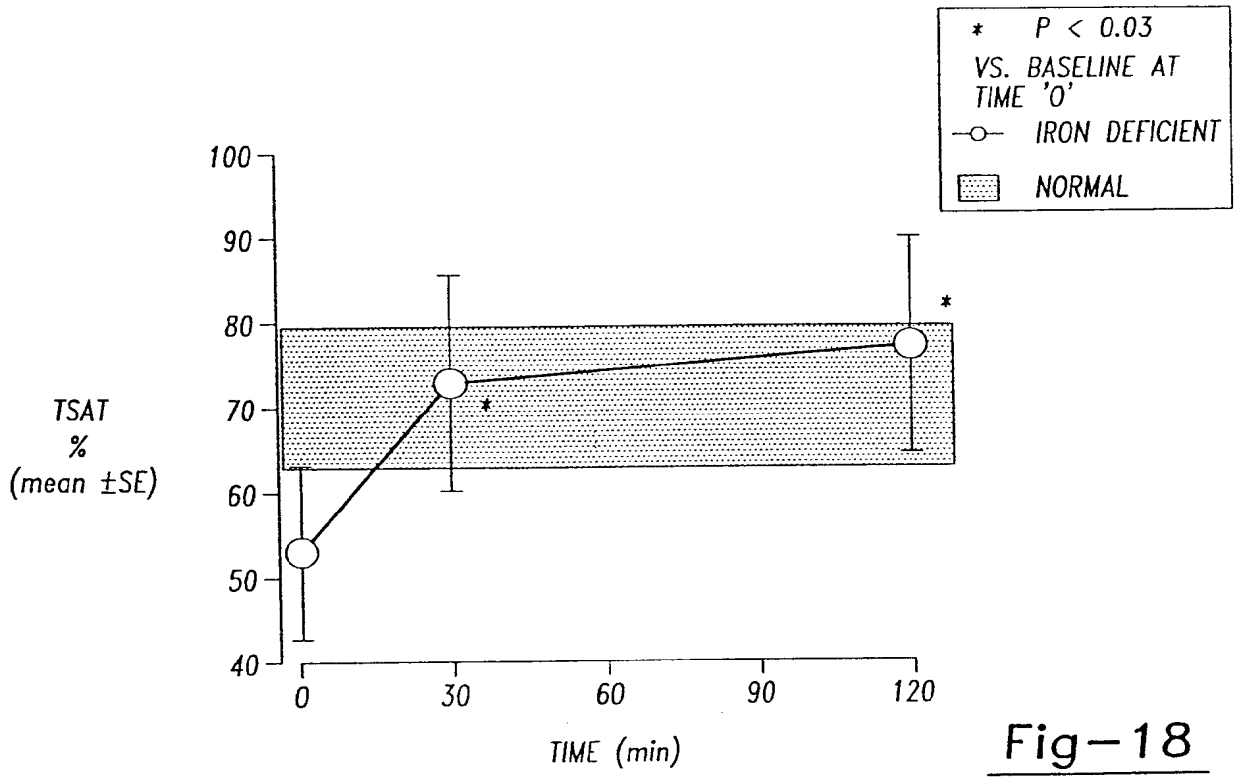


Fig-16


350
300
250
200
TIBC
µg/dl
(mean ±SE)
IRON DEFICIENT
NORMAL
0
30
60
90
200
TIME (min)

Fig–17


* P < 0.03
VS. BASELINE AT TIME '0'
IRON DEFICIENT
NORMAL
100
90
80
70
60
50
40
TSAT
%
(mean ±SE)
0
30
60
90
120
TIME (min)

Fig–18

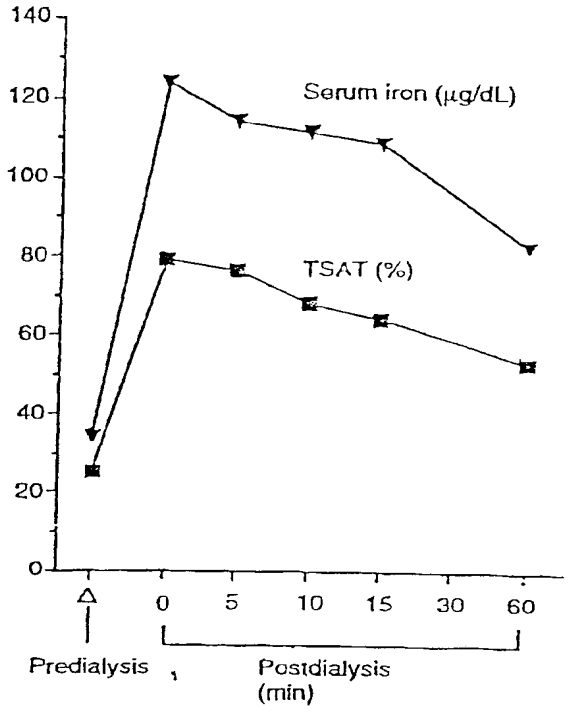

140
120
100
80
60
40
20
0
Serum iron (μg/dL)
TSAT (%)
0
5
10
15
30
60
Predialysis
Postdialysis
(min)

Fig-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/17311

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/295; B01D 61/24
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/646,647; 424/603, 646, 647, 648; 514/23, 502, 814; 604/27, 28, 29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,906,978 A (ASH) 25 May 1999 (25.05.99), see entire document.	1-3, 7-14, 19-20
Y	US 5,108,767 A (MULCHANDANI et al) 28 April 1992 (28.04.92), see entire document.	6, 16-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
27 AUGUST 2000

Date of mailing of the international search report
20 SEP 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
SUN UK KIM
DEBORAH THOMAS
PARALEGAL SPECIALIST
Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/17311

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

210/646,647; 424/603, 646, 647, 648; 514/23, 502, 814; 604/27, 28, 29